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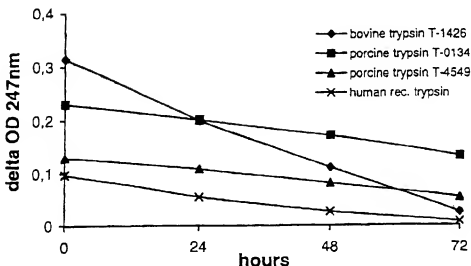
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(54) Title: LIVE VACCINE AND METHOD OF MANUFACTURE



(57) Abstract: The invention relates to a simple and efficient process for isolating viruses from various sources and for producing live attenuated influenza vaccines in a serum-free Vero cell culture under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or prevented. The process does not require purification of the virus-containing supernatant harvested from the cell culture nor post-incubation treatment of the viruses for HA activation. The invention further relates to influenza A and B master strain candidates and to vaccines made thereof.

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LIVE VACCINE AND METHOD OF MANUFACTURE

TECHNICAL FIELD

- 5 The present invention is in the field of virology and vaccine development and relates to an improved method of manufacture of a viral vaccine, particularly of a whole-virus vaccine, preferably of an attenuated live vaccine and to vaccines obtainable by the method.

10 BACKGROUND OF THE INVENTION

The influenza hemagglutinin (HA) antigen is the major target for the protective immune responses of a host to the virus.

- A common practice of recovering new viral isolates involves recovery from a
15 nasal or throat swab or from a similar source, followed by cultivation of the isolates in embryonated chicken eggs. The virus adapts to its egg host and large scale production of the virus can be carried out in eggs. Such conventional methodology involving embryonated chicken eggs to produce Influenza vaccine is, however, extremely cumbersome, involving the handling of many thousands
20 of eggs per week as well as extensive purification of the virus suspension derived from the allantoic fluid to ensure freedom from egg protein.

- Another disadvantage in the use of chicken embryos for virus production lies in the fact that this substrate strongly favors the selection of virus variants that
25 differ in their antigenic specificity from the wildtype virus and not rarely results in viruses that may not be suitable for vaccine production due to their altered phenotypes including, for instance, considerable reduction in immunogenicity.

- Many attempts have therefore been undertaken in the art to utilize standard
30 tissue culture technology with established mammalian cell lines, such as MDCK (Madin-Darby Canine Kidney) or Vero (African Green Monkey Kidney) cells, for virus production, particularly influenza virus production.

- One of the difficulties in growing influenza strains in tissue cell culture arises
35 from the necessity for proteolytic cleavage of the influenza hemagglutinin in the host cell. Cleavage of the virus HA precursor into the HA1 and HA2 subfragments, although not necessary for the assembly of the viral elements to

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form a complete virion, is required, however, to render the virion infective, i.e. to enable it to infect a new cell.

- It has been reported (e.g. Lazarowitz et al., "Enhancement of the Infectivity of Influenza and B Viruses by Proteolytic Cleavage of the Hemagglutinin Polypeptide", Virology, 68:440-454, 1975) that the limited replication of several influenza A strains in standard cell cultures could be overcome by the addition of proteases like trypsin to the tissue culture medium. Yet, there remained difficulties in some cases, for instance when using Vero cells.
- 10 Kaverian and Webster (J Virol 69/4:2700-2703, 1995) report that in Vero cell cultures, and less pronounced in MDCK, swine kidney, or rhesus monkey kidney cell cultures, the trypsin activity in the medium rapidly decreased from the onset of incubation resulting in the failure of virus accumulation in the medium due to the lack of production of a sufficient number of infective virions. They
- 15 concluded that a trypsin inhibiting factor was released from the Vero cells. They further showed that by repeated addition of trypsin reproduction of virus could be resumed and maintained for a number of reproduction cycles resulting in a much better virus yield.
- 20 Another way for efficient vaccine production was reported in US 5,753,489 wherein serum-free medium was used for virus propagation in a number of different mammalian cells including MDCK and Vero cells. The method disclosed therein comprises growing vertebrate cells in serum-free medium, infecting the
- 25 cell culture with a virus, incubating the cell culture infected with the virus, removing a portion of the virus-containing medium and contacting this portion with a protease, thereafter adding to that portion a protease inhibitor and returning that portion to the cell culture. It is preferred therein to provide the steps of growing, infecting and incubating in a first vessel and the steps of
- 30 trypsin-contacting and inhibitor-adding are performed in a second vessel connected with the first vessel in a loop so that the steps can be performed in a closed cycle. This system allows to use trypsin or other proteolytic enzymes at much higher concentrations than those normally tolerated by cells in culture.
- 35 EP 0870508 reports a method to produce a viral antigen vaccine comprising infecting an animal cell line, optionally a Vero cell line, with virus, propagating virus in the cell culture, adding a nuclease enzyme to the cell culture shortly

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- before the end of virus propagation to digest nucleic acid material released from the lysing host cells into the medium, harvesting the virus and obtaining viral antigens thereof by extraction in order to make the viral antigen vaccine. The patent is silent with regard to the kind of nutrient medium used for virus
- 5 propagation and also with regard to the addition of a protease, usually required for the final processing of influenza virus hemagglutinin to get infectious virus. The method further requires various purification steps for providing a ready-for-use vaccine preparation.
- 10 It is known, however, that the nature the host substrate as well as the composition of the nutrient medium used for virus propagation may significantly affect immunogenicity and antigenicity of the virus progeny obtained therewith. Particularly, serum-containing media may not only decrease antigenicity of viral progeny but additionally may decrease protease activity in the medium, hence
- 15 inhibit virus maturation, and subsequently require expensive steps of purification.

SUMMARY OF THE INVENTION

- 20 The present invention overcomes the drawbacks of the prior art. It relates to a simple and efficient process for isolating viruses from various sources and for producing viral progeny for use as vaccines, particularly live attenuated influenza vaccines, in under conditions where alterations in the surface antigens of the virus due to adaptive selection are minimized or entirely prevented.
- 25 It is also an object of the present invention to provide for a method for the production of viruses, particularly influenza viruses, that yields viral progeny that selectively agglutinates human erythrocytes but not chicken erythrocytes, and that preferably has antigenic properties identical with those of the initially
- 30 inoculated virus strain, e.g. a primary clinical wildtype isolate.

- In a preferred embodiment, the nucleic acid sequence of the HA gene and optionally of the NA gene of the propagated virus is identical with the one of the initially inoculated strain (e.g. an epidemic strain, primary clinical isolate of
- 35 an infected patient).

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It is another object of the invention to provide a method for efficient production of a whole-virus vaccine, particularly a live attenuated vaccine, in a single step procedure that does not require any chromatographic or other purification steps of the virus suspension harvested from the cell culture supernatant by
5 centrifugation, particularly no protein separation or purification steps.

It is yet another object of the invention to provide attenuated, cold adapted and temperature sensitive influenza A and B strains and vaccines made thereof.

10 BIREF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a Vero cell culture.

15 Fig. 2 is a graphic illustration of the time course of trypsin inactivation in the supernatant of a MDCK cell culture.

DETAILED DESCRIPTION OF THE INVENTION

20 Comparative experiments using embryonated eggs, MDCK and Vero cells clearly proved that the initially inoculated virus is likely to undergo antigenic alteration during growth on any one of these substrates

Our experiments confirmed that the alterations are least or even absent for
25 influenza virus strains grown on Vero cells in serum-free medium. Moreover, it turned out that influenza A viruses, at least strains of the H3N2 subtype, when multiplied on Vero cells in serum-free and protein-free medium exhibit a selectivity for agglutination of human erythrocytes but not for chicken erythrocytes. Also, they did not grow on eggs. This was a first indication that
30 these Vero-grown viruses might be more identical with the wildtype virus of the corresponding clinical isolate than the ones grown on MDCK cells or eggs.

Indeed, comparison of the HA and NA gene sequences of wildtype isolates obtained from nasal swabs with the ones of the same viruses after growth on
35 Vero and MDCK cells, respectively, revealed alterations in the HA or NA of MDCK-grown viruses relative to the HA or NA of the swab isolates or of the Vero-grown viruses or of both the swab isolates and the Vero-grown viruses.

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Moreover, experimental data obtained from immunizations of ferrets with Vero- and MDCK-grown wildtype viruses indicate a far stronger virulence of the Vero-grown viruses compared to the MDCK-grown viruses. Also, the immunogenicity of the Vero-grown viruses tested in an animal trial on macaques was

- 5 demonstrated to be significantly superior to the one of the viruses grown on MDCK cells or eggs.

These findings together provide strong evidence for the hypothesis that the process for the multiplication and propagation of viruses according to the

- 10 present invention as hereinafter described in more detail yields viruses that are either unaltered compared to the initially inoculated (e.g. wildtype) virus or are modified to only a minor extent.

It is not only the avoidance of antigenic alterations that makes the present

- 15 process of virus multiplication so unique, but it is also its striking simplicity which makes it extremely suitable for large scale industrial vaccine production.

Further experiments have shown that the source of trypsin (or trypsinogen) may be one additional factor that influences the overall yield of infective virions.

- 20 Indeed, while the methods known in the art (e.g. Kaverin and Webster, J Virol 69/4:2700-2703, 1995; or US 5,753,489) use either repeated addition of trypsin (Kaverin and Webster) or high trypsin concentrations (US 5,753,489), the process according to the present invention applies only half or less of the trypsin concentrations reported in the prior art. Moreover, a single addition of as
25 little as 0.5 - 10 µg, preferably 2 - 5 µg trypsin per ml to the cell culture medium prior to or at the beginning of incubation of the infected host cells is sufficient to reach optimal infective virus titers. Inactivation experiments revealed that porcine or human recombinant trypsin are far less susceptible to inactivation by Vero or MDCK cells than bovine trypsin. Since bovine trypsin
30 most commonly used in the art it is rather likely that prior art literature unless explicitly mentioning another trypsin source, implicitly refers to bovine trypsin only. This would also help to explain the modes and concentrations of trypsin application recited, for instance, in Kaverin et al. and in US 5,753,489.

- 35 Using porcine or human rec trypsin or trypsinogen for initially supplementing the serum-free medium for Vero cell cultures according to the present invention therefore allows to use extremely low trypsin or trypsinogen concentrations and

thus prevents the need of labor-intensive and costly purification steps after harvesting of the virus-containing supernatant.

- Another step that contributes to make the present process simple and therefore attractive to vaccine manufacturers is the addition of a single dose of highly active endonuclease to the cell culture medium prior to or at the beginning of incubation of the infected Vero cells for virus propagation. This endonuclease, preferably BenzonaseTM, is added once to the medium at a very low initial concentration of 2 - 30, preferably 5 - 15, Units per ml of medium and effectively clears the cell culture medium from free DNA and RNA originating mainly from the lysing or lysed host cells. The residual Benzonase enzyme concentration in the ready-for-use vaccine preparations obtained from the centrifuged supernatant remains at 5 ng or less per dose.
- BenzonaseTM is a trademark of Nycomed Pharma A/S Denmark and relates to an extracellular unspecific endonuclease obtained from *Serratia marcescens*. Benzonase is a genetically engineered endonuclease which degrades both DNA and RNA strands in many forms to small oligonucleotides. It promotes quick reduction of the viscosity of cell lysates, which facilitates ultracentrifugation. Also, because the addition of exogenous enzymes such as the protease (e.g. trypsin or trypsinogen) and nuclease (e.g. Benzonase) occurs once at the beginning of the virus propagation phase this saves plenty of time that the state-of-the-art methods require for post-incubation treatment of the virus-containing culture supernatant (e.g., HA activation, RNA/DNA digestion, protein purification, etc.). Surprisingly, it turned out that the early addition of either or both of protease (e.g. trypsin or trypsinogen) and nuclease (e.g. Benzonase) to the virus-infected Vero-cell culture had no negative implications on the virus yield, which is

probably due to the very low enzyme concentrations applicable in the process of the present invention.

- The present process of virus propagation is useful for the multiplication of various kinds of viruses, particularly influenza A viruses of the H3N2 subtype, but is also suitable for the isolation and reproduction of any epidemic or laboratory influenza virus strain, regardless of the kind of virus inoculum (e.g., blood serum sample, nasal wash, nasal swab, pharyngeal swab, saliva, etc.). Using the principles of this process, a number of influenza A and B vaccines has been produced which are part of the present invention and which are characterized in more detail in the subsequent Examples. Also, protective efficacy as well as vaccine safety have been confirmed for the vaccines made according to the present invention, as will be demonstrated in the Examples.

- The term "protein-free" or "free of non-serum proteins" as used herein in connection with the method of virus multiplication or propagation according to the present invention shall mean free of any functionally active protein. It shall not exclude, however, non-functional peptides as may originate from protein hydrolysates such as yeast extract or soya extract. Unless stated otherwise, the term "protein-free" shall neither exclude the presence of a protease and a nuclease enzyme at the concentrations disclosed and claimed herein.

- In a preferred embodiment, the present invention relates to a simple, reliable and highly economic method for the manufacture of a whole-virus vaccine, preferably of an attenuated live vaccine, comprising the steps of:
- inflecting African Green Monkey Kidney (Vero) cells with a desired virus, wherein the Vero cells have been grown in and separated from a serum-free medium that is also free of non-serum proteins;
 - combining the infected cells with a suitable serum-free cell culture medium that is also free of non-serum proteins except for a protease and a nuclease; and
 - incubating the cells in the presence of said protease and said nuclease to allow for production of infectious virus and, simultaneously, for digestion of nucleic acid material released to the cell culture medium;
 - harvesting infectious virus by collecting virus-containing supernatant obtained from centrifugation of the cell culture; and

e) preparing a vaccine thereof comprising subjecting the virus-containing supernatant to at least one processing step selected from the group consisting of filtering, concentrating, freezing, freeze-drying, and stabilizing by addition of a stabilizing agent.

5

It is preferred that the virus used for propagation has never had any contact to a host substrate other than a Vero cell line. This will ensure best results with regard to immunogenic and antigenic identity of the initial virus (e.g. nasal swab isolate) and the viral progeny obtained after propagation.

10

It is also preferred that the virus used for propagation, particularly for the manufacture of a whole-virus vaccine, preferably an influenza attenuated live vaccine, is an influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ Δ NS 87, A/Sing/1/57ca/ Δ NSPR8,

15 A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, B/Vienna/99/ca37 and any attenuated variants and reassortants derived from any one of these strains. The genetic characteristics of the preferred virus strains, e.g. master strains, are disclosed in full detail in the subsequent Examples.

20 In another embodiment, the present invention refers to a whole-virus vaccine itself, preferably to an attenuated live vaccine, which in its ready-for-use form comprises essentially unmodified, optionally filtered and/or concentrated, virus-containing supernatant of a serum-free and protein-free Vero cell culture used for production of said virus. It is particularly preferred that the vaccine is
25 produced according to the method of the present invention as disclosed and claimed herein.

This "one-step" vaccine, which does not require further processing, e.g., purification steps other than centrifugation and/or conventional filtration (i.e. not gel filtration), is compliant with the requirements for FDA approval.

30

The term "essentially unmodified" as used herein with regard to virus-containing supernatant in vaccine preparations according to the present invention shall refer to the composition of the supernatant as is at the time of harvesting the propagated virus, i.e. to the composition of the soluble components and

35 ingredients present in the liquid phase of the supernatant. Minor alterations of the composition of ingredients as may occur due to steps of, for example, filtration, sterile filtration, centrifugation, concentration, drying, or freeze-drying

of the virus-containing supernatant, shall be regarded as falling within the scope of "essentially unmodified". Also, the term shall not exclude the presence of preserving and/or stabilizing agents usually applied in the art to vaccine preparations.

5

The whole-virus vaccines of the present invention may be used for the prophylactic or therapeutic treatment of viral infections, particularly of influenza virus infections. They may be administered as known in the art, e.g. intravenously, subcutaneously, intramuscularly or, most preferably, intranasally.

10 The virus strains disclosed herein and the vaccines made thereof may, however, also be used as vectors or shuttles to present heterologous antigens to the immune system, e.g. antigens of viral envelope proteins such HIV-1 or hepatitis antigens.

15 Further preferred embodiments are defined in the dependent claims.

In order that the invention described herein may be more fully understood, the following Examples are set forth. They are for illustrative purposes only and are not to be construed as limiting this invention in any respect.

20

Example 1: Virus production

Cultivation of Vero /SF (=serum-free) cells:

25 SF-Medium: DMEM (Biochrom F0435), Ham's F12 (Biochrom F0815), 5mM L-Gln, 0.1% SF-supplement (a) or (b); antibiotics (only for first passage of virus isolation).

SF-Supplement: protein hydrolysate of non-animal origin, without functional proteins such as insulin, transferrin or growth factors:

30 a) 62.5 g hy-soy/UF, Quest 5X59100, to 500 g HQ-water, filtered with PES 0.2 µm filter;
b) 12.5 g hy-pep 1510, Quest, to 100 g HQ-water, filtered with PES 0.2 µm filter.

35 The content of a deep frozen (liquid nitrogen) disinfected (70% ethanol) ampule of WCB Vero cells was thawed and added to 9 ml of cold serum-free (SF) medium in a 10 ml tube and centrifuged for 10 min at 1000rpm (170 g). The

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pellet was resuspended in SF-medium to a total of 30 ml, transferred to a 80 cm² Roux bottle and incubated at 37°C and 7%CO₂ for at least 15 min. Thereafter, the medium was removed and the cells were washed with approx. 0.1 ml/cm² PBS def.(= PBS without Ca²⁺ and Mg²⁺). Addition of
5 trypsin/EDTA-solution (8-10 µl/cm²; 0.1% trypsin / 0.02% EDTA-solution) and incubation at room temperature for about 3 min. Detaching by gently pushing the Roux bottle against palm of the hand, addition of SF-medium and trypsin inhibitor (Sigma, T6522) at a quantity of about 1/5 of volume of the trypsin/EDTA solution. Repartition of the cell suspension to Roux bottles or
10 roller bottles, incubation at 37°C and 9% CO₂.

MDCK cells were grown in DMEM/Ham's F12 + 2% FCS (heat inactivated); embryonated hen eggs were 11-12 days old and of SPF (specific pathogen free) origin.

15

Propagation of virus strains:

Old medium from roller bottles containing Vero cells was removed and cells were infected with virus by addition of 5 ml virus suspension in SF-medium to
20 each roller bottle, resulting in an MOI (multiplicity of infection) of approximately 0.01. After incubation for 45 minutes at 33°C the virus inoculum was removed with a pipette. 90ml of SF-medium supplemented with 0.5 - 10, preferably 2 - 5 and most preferably 2 µg/ml porcine trypsin (supplier: AvP) or human recombinant trypsin or trypsinogen (own production) and 0.5 g/l sodium
25 bicarbonate were added to each roller bottle and the bottles incubated at 33°C and 5% CO₂. For the production of attenuated live vaccine samples for use in animal testing and in human clinical trials the SF-medium was supplemented with trypsin and, additionally, with BenzonaseTM at a concentration of 2 - 30, preferably 5 - 15, and most preferably 10 Units of BenzonaseTM per ml of
30 medium. Virus was harvested after 64 hours post infection by centrifugation of the culture supernatant for 5 min at 4000 rpm (3000g) at 10°C in 50 ml-tubes. The supernatant was pooled for each virus strain and stored at +4°C. Aliquots thereof were used for vaccine testing.

35 For storage purposes the virus preparations may be freeze-dried and stabilizer such as, for example, trehalose and lactalbumin enzymatic hydrolysate in HEPES buffer may be added. Reconstitution may be done with sterile water.

Example 2: Comparison of trypsin inactivation in cell cultures

Table 1: Trypsin inactivation in Vero vs. MDCK cell culture

	Vero / MDCK			
	0 h	24 h	48 h	72 h
bovine trypsin	0.314/0.314	0.199/0.239	0.110/0.201	0.026/0.203
porcine trypsin (high)	0.230/0.230	0.201/0.206	0.171/0.209	0.133/0.201
porcine trypsin (low)	0.129/0.129	0.108/0.118	0.081/0.099	0.054/0.116
human rec trypsin	0.097/0.097	0.054/0.088	0.026/0.080	0.008/0.076

- 5 Supernatants obtained from uninfected Vero cell cultures (grown in SF medium as described in Example 1) and MDCK cell cultures (grown in FCS-supplemented medium as described in Example 1) were tested for their capacity to inactivate trypsin of different origin that has been added to the supernatant at time = 0 h at equal concentrations each. Porcine trypsin has been applied in two different
- 10 qualities (obtained from different manufacturers), i.e. with high or low activity. The results are presented in Table 1 and in Figures 1 and 2.

The data unambiguously show that bovine trypsin is rapidly inactivated in Vero cell culture supernatant and less rapidly in MDCK cell culture supernatant.

- 15 Porcine and human rec trypsin (manufactured in our laboratories) remain fully active in MDCK supernatants while they are gradually inactivated in Vero supernatants at approximately half or less of the velocity of bovine trypsin inactivation. The difference of the porcine trypsins tested is only in the starting OD-level at 247 nm, while the inactivation characteristics are essentially
- 20 identical for both lots of porcine trypsin.

Example 3: Comparison of various viral properties after growth on different host cell substrates

- 25 Virus propagation was carried out as described in Example 1 for the different host cell substrates. Each of the seven isolates recovered on Vero cells was reactive with human erythrocytes but not with chicken erythrocytes and none of them accumulated in embryonated eggs. On the other hand, all isolates recovered on MDCK cells were reactive both with chicken and human
- 30 erythrocytes and were capable of growing in eggs. Although these differences were not seen in influenza A viruses of the H1N1 subtype nor in influenza B

isolates (see subsequent Tables 3 and 4), it may nevertheless be assumed that cultivation of influenza viruses on Vero cells will maintain antigenic properties more properly than cultivation on other substrates.

5 Table 2: Characteristics of H3N2 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs
			chicken erys	human erys	
A/47/96	A/Johannesburg/33/94	Vero	-	+	-
		MDCK	+	+	+
A/7729/98	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1143/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1144/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1179/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1180/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+
A/1182/99	A/Sydney/5/97	Vero	-	+	-
		MDCK	+	+	+

From the data in Table 3 it appears that H1N1 influenza viruses may be less susceptible to adaptive selection, as the Vero and MDCK-grown isolates do not exhibit significant differences in their hemagglutination characteristics nor in their HA sequences. A similar conclusion may be drawn for the B isolates listed in Table 4.

The clinical starting material (e.g. serum samples, swabs) for virus isolation and replication was primarily obtained from:

- 15 1. Institute of Virology, Vienna, Austria (Prof. F. Heinz) 1995/96, 1996/97
2. Unité de Génétique Moléculaire des Virus Respiratoires, Institute Pasteur, Paris, France (Prof. S. van der Werf) 1996/97
3. Public Health Laboratory Service, London, UK (Dr. M. Zambon) 1996/97
4. Laboratoire Central de Virologie, Hôpitaux Universitaires de Genève, Geneva, Switzerland (Dr. W. Wunderli) 1996/97, 1997/98

5. Virus Unit, Queen Mary Hospital, Hong Kong (Dr. W.L. Lim) 1997/98

Table 3: Characteristics of H1N1 viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		225
A/5389/95	A/Bayern/7/95	Vero	+	+	+	D
		MDCK	+	+	+	D
A/1035/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	G
		Swab				D
A/1131/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Swab				D
A/1134/98	A/Beijing/262/95	Vero	+	+	+	D
		MDCK	+	+	+	D
		Egg	+	+	+	n.t.
		Swab				D

5

Tabelle 4: Characteristics of B viruses isolated from clinical material on Vero/SF cells

Isolate number	Antigenically related to	Isolated on	HA titer with		Growth in eggs	Changes in HA1 at position
			chicken erys	human erys		198
B/4291/97	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/1/99	B/Beijing/184/93	Vero	+	+	+	T(g.s)
		MDCK	+	+	+	T(g.s)
		EGG	+	+	+	A
		Swab				T(g.s)

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B/110/99	n.t.	Vero	+	+	+	identical
		MDCK	+	+	+	
B/147/99	n.t.	Vero	+	+	+	identical
		MDCK	+	+	+	
B/156/99	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	
B/157/99	B/Beijing/184/93	Vero	+	+	+	identical
		MDCK	+	+	+	

Table 5: Amino acid changes in HA, NA and M proteins of H3N2 influenza viruses isolated on different host systems

Isolate number	Changes at positions								
	HA						NA		M
	128	129	229	133	218	220	136	151	
A/47/96 Vero	T(g.s)								
A/47/96 MDCK	A								
A/7729/98 Vero		E	R						
A/7729/98 MDCK		G	K						
A/1143/99 Swab				N(g.s)	G		n.t	n.t	n.t
A/1143/99 Vero				N(g.s)	G			D	
A/1143/99 MDCK				D	E			G	identical
A/1144/99 Swab						R	n.t		n.t
A/1144/99 Vero						R	identical		identical
A/1144/99 MDCK						G			
A/1179/99 Swab	identical						n.t		n.t
A/1179/99 Vero							identical		identical
A/1179/99 MDCK									
A/1180/99 Swab	identical						n.t	n.t	n.t
A/1180/99 Vero							Q		
A/1180/99 MDCK							R		identical
A/1182/99 Swab	identical						n.t		n.t
A/1182/99 Vero							n.t		n.t
A/1182/99 MDCK							n.t		n.t

5

The results show that with some isolates there was no alteration of the HA sequence of Vero or MDCK propagated viruses over the HA sequence directly obtained from the swab material by PCR amplification. In some other isolates

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grown on MDCK cells the HA and/or NA sequences were deviating from the corresponding sequences obtained on Vero cells. The Vero-derived viruses did not show, however, any deviations in the HA sequence over the HA sequence of the swab isolates, where determined.

5

Table 6: Immunogenicity of Vero-, MDCK- and Egg-derived viruses for macaques

Animal number	Virus for immunization	Dose, PFU/ml	Serum HI titers
96	A/Vienna/47/96 V	5×10^4	256
88	A/Vienna/47/96 V	5×10^4	128
15	A/Vienna/47/96 V	1.0×10^6	128
95	A/Vienna/47/96 V	1.0×10^6	256
93	A/Vienna/47/96 M	1.0×10^6	16
128	A/Johannesburg/33/94 E	5×10^6	32
110	A/Vienna/157/97 V	5×10^4	128
78	A/Wuhan/359/95 E	5×10^6	32

The Macaques were immunized i.n. in the absence of anesthesia with 1 ml of virus suspension

10 V – Vero- isolated virus

M - MDCK -isolated viruses

E - egg isolated viruses

Table 7: Virulence of Vero- and MDCK- derived variants of A/Vienna/47/96 wt virus for ferrets

15

Viruses	Virus dose, PFU/ml	Number of animals with fever on day		
		1	2	3
A/Vienna/47/96 Vero	2×10^2	FF	FFF	
	1×10^3	FFF	FFF	
A/Vienna/47/96 MDCK	5×10^2			
	5×10^3		FF	
	5×10^4	FF	F	F

Animals were immunized i.n. under ether narcosis with 1 ml of virus suspension.

N- normal temperature from 38.1 °C to 39.9 °C;

F- fever, more than 40.0 °C.

The most surprising, yet important result in Table 6 is the very low immunogenicity of MDCK-derived A/Vienna/47/96 virus compared with the corresponding Vero-derived virus. It is no particular surprise that the egg-derived viruses show only poor immunogenicity.

5

Similarly, the results listed in Table 7 indicate that Vero-derived viruses are less, if at all, altered by adaptive selection on their host substrate in comparison to MDCK-derived viruses. This means that relative to the MDCK-derived viruses the Vero-derived viruses maintain more or even all of the immunologically

10 relevant, particularly antigenic, properties of the original virus.

Example 4: Vaccine production with preferred strains

The process described in Example 1 was also used for the production of vaccine samples for animal testing and human clinical studies. It is understood that the process of virus propagation described therein also encompasses variations that could be suggested or applied by a person of ordinary skills in the art without inventive input and as long as the variations do not change the sense of the present invention as described herein and in the claims.

20

Vaccine samples containing one or more of the preferred influenza A or B wildtype strains, master strains or reassortant strains (that are subsequently described in more detail) were exclusively produced using the continuous Vero cell line as the host cell system (unless for purposes of comparison with samples obtained from other host substrates) in serum-free medium additionally supplemented with the nutritional ingredients and enzymes as described in Example 1.

25

Some methods suitable for modifying wildtype viruses including the methods of attenuation (e.g., temperature sensitivity), cold adaptation and reassortment are known in the art and extensively reviewed, for instance, in WO 99/64068.

30

Further characteristics of the two most preferred influenza A and B master strain candidates useful for attenuated live vaccine production, e.g., by 6/2 reassortment with the HA and NA genes of actual epidemic influenza viruses recommended by the WHO, are given in the following Tables 8 - 13.

35

Table 8: Characteristics of master strain candidates for live influenza vaccines

	Influenza A <i>A/Singapore/1/57/ca</i> H2N2	Influenza B <i>B/Vienna/1/99/ca</i>
Passage history	A/Singapore/1/57 wt egg derived H2N2 20 passages at 37°C on Vero/SF cells 25 passages at 25°C on Vero/SF cells	B/Vienna/1/99 wt Vero derived 1 additional passage at 33°C on Vero/SF cells 22 passages at 25°C on Vero/SF cells
Method of attenuation	Serial passages at optimal and suboptimal temperature on heterologous system	Serial passages at optimal and suboptimal temperature on heterologous system
Phenotypic markers	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs	temperature sensitive (ts) cold adapted (ca) very low reproduction in mouse lungs
Genotypic markers	Mutations: 13 (8 coding) PB2 3 (2 coding) PB1 2 (1 coding) PA 4 (3 coding) NP 1 M 2 (2 coding) NS 1	Mutations: 5 (3 coding) PB2 0 PB1 1 PA 0 NP 2 (1 coding) M 1 NS 1

Table 9: Full Sequence of the 8 genome segments and of the 10 corresponding proteins of strain A/Singapore/1/57/ca

A/Singapore/1/57/ca (H2N2)			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 1	PB2	SEQ ID No. 9
2	SEQ ID No. 2	PB1	SEQ ID No. 10
3	SEQ ID No. 3	PA	SEQ ID No. 11
4	SEQ ID No. 4	HA	SEQ ID No. 12
5	SEQ ID No. 5	NP	SEQ ID No. 13
6	SEQ ID No. 6	NA	SEQ ID No. 14
7	SEQ ID No. 7	M1	SEQ ID No. 15
		M2	SEQ ID No. 16
		NS1	SEQ ID No. 17
		NS2	SEQ ID No. 18
8	SEQ ID No. 8		

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ca - cold adapted

It shall be noted, however, that the genome segments No. 4 and 6, i.e., the HA and NA genes, are not required to characterize the influenza A master strain candidates, because these genes will be exchanged for the corresponding genes of actual epidemic influenza viruses (as mentioned hereinbefore). The features important for the safety of a vaccine, e.g. temperature sensitivity, or features that allow intranasal administration of a vaccine, namely cold adaptation (because the average temperature in a nose is lower than the usual body temperature), are primarily caused by mutations in the remaining 6 genome segments.

The following Table 10 lists the mutations in the genome segments of A/Singapore/1/57/ca compared to the corresponding wildtype strain A/Singapore/1/57/wt.

Table 10: Mutations in the genome segments of attenuated, temperature sensitive, cold adapted influenza strain A/Singapore/1/57/ca compared to A/Singapore/1/57/wt strain

RNA segment	Length (n'ds)	Nucleotides changed			Protein	Length (aa)	Amino acids changed		
		position	wt	ca			position	wt	ca
1	2341	252	a	g	PB2	771	-	-	-
		581*	t	c			185	I	T
		1046*	g	t			340	R	I
2	2341	1279*	t	a	PB1	757	419	L	I
		1965	a	c			-	-	-
3	2233	707*	a	t	PA	716	228	I	N
		1425	t	a			-	-	-
		1537*	a	g			505	V	I
		1819*	g	c			598	Q	E
5	1565	210	g	a	NP	506	-	-	-
7	1027	327*	g	a	M1	252	101	R	K
		499*	g	c			158	Q	R
8	890	813	a	g	M2	97	-	-	-
					NS1	237	-	-	-
					NS2	121	-	-	-

20 Total number of mutations - 13 (8 coding)

* coding mutations

Preferred variants of A/Sing/1/57/ca comprise the ones listed in the following Table 11, wherein "Δ" means "del" or "delta" and stands for a mutant that contains at least one "deletion" in its NS gene segment.

5 Table 11: Preferred variants of A/Sing/1/57/ca

	A/Sing/1/57/ca	Sing ca/ ΔNS 87	Sing ca/ ΔNSPR8	Sing ca/ NS124PR8
PB2 (Sing ca*)				
PB1 (Sing ca*)				
PA (Sing ca*)				
HA				
NP (Sing ca*)				
NA				
M1,2 (Sing ca*)				
NS1,2 (Sing ca*)				
NS1,2 (PR8**)				
Phenotypes				
ca	+	+	+	+
ts	+	+	+	+
IFN-induct.	—	+ / —	+	+
IFN-sensit	—	+	+	—

* genome segment originating from A/Singapore/1/57/ca

** genome segment originating from influenza A/PR8/34

ca - cold adapted; ts - temperature sensitive;

aa - amino acid(s)

- 10 IFN-induct. - strain causes interferon release in host substrates that are able of IFN production, as well as in animal or human immune systems upon administration.

IFN-sensit. - strain is sensitive towards interferon; replication in IFN producing systems is reduced or stopped.

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Sing ca/ Δ NS 87 - strain A/Singapore/1/57/ca containing deletion of 87 amino acids in NS1 gene at aa position 36-123.

Sing ca/ Δ NSPR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 (herein also abbreviated "PR8") which contains a deletion of the entire NS1 gene.

Sing ca/NS124PR8 - strain A/Singapore/1/57/ca containing the NS gene segment from A/PR8/34 which contains a stop codon at aa position 124 of the NS1 gene.

+/- means that the phenotype needs further clarification and can not yet be unambiguously defined.

The following Tables 12, 13 and 13A refer to preferred influenza B master strain candidates and to variations and reassortants, respectively, thereof.

15 Table 12: Full Sequence of the 8 genome segments and of the 11 corresponding proteins of strain B/Vienna/1/99/ca

B/Vienna/1/99/ca			
RNA segment	Nucleotide sequence (cDNA)	Protein	Amino acid sequence
1	SEQ ID No. 19	PB2	SEQ ID No. 27
2	SEQ ID No. 20	PB1	SEQ ID No. 28
3	SEQ ID No. 21	PA	SEQ ID No. 29
4	SEQ ID No. 22	HA ₀	SEQ ID No. 30
5	SEQ ID No. 23	NP	SEQ ID No. 31
6	SEQ ID No. 24	NB NA	SEQ ID No. 32 SEQ ID No. 33
7	SEQ ID No. 25	M1 BM2	SEQ ID No. 34 SEQ ID No. 35
8	SEQ ID No. 26	NS1 NS2	SEQ ID No. 36 SEQ ID No. 37

ca - cold adapted

The original strain B/Vienna/1/99 was isolated on Vero cell culture grown with serum-free medium in February 1999 in Vienna, Austria from a 12 year old female with acute influenza. It was rated as B/Beijing/184/93-like by the Center for Disease Control (CDC), Atlanta, USA. After an additional passage at 33°C the wildtype strain - designated as B/Vienna/1/99 wt - was attenuated by 22

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serial passages at 25°C using the same cell culture system. The plaque purification was done at 25°C for the first and at 33°C for the following four rounds. The derived plaque purified clone was amplified and stored at -70°C, designated as B/Vienna/1/99 ca or briefly BV22. The identity as a

- 5 B/Beijing/184/93-like virus was confirmed by HI-assay with standard anti-serum from NIBSC.

Table 13: Mutations in B/Vienna/1/99/ca (=BV22) compared to B/Vienna/1/99/wt (BVie) 1. passage on Vero/SF

Segment (length in nucleotides)	Nucleotides changed			Protein (length in amino acids)	Amino acids changed		
	Posi- tion	BVie	BV22		Posi- tion	BVie	BV22
1 (2396)	-	-	-	PB2 (770)	-	-	-
2 (2369)	594	T	C	PB1 (752)	-	-	-
3 (2305)	-	-	-	PA (726)	-	-	-
4 (1882)	457	G	A	HA ₀ (584)	142	A	T
	1299	G	T		422	K	N
	1595	G	A		521	G	E
5 (1844)	128	C	T	NP (560)	23	S	F
	330	T	C		-	-	-
6 (1557)	-	-	-	NB (100)	-	-	-
	823	G	A	NA (466)	257	R	Q
	1135	T	C		361	I	T
7 (1190)	-	-	-	M1 (248)	-	-	-
	831	A	G	BM2 (109)	21	M	V
8 (1097)	116	G	A	NS1 (281)	25	A	T
	-	-	-	NS2 (122)	-	-	-

10

Table 26: Characterization of B/Vienna/1/99 wt according to Los Alamos National Library influenza database (db) (Web-address: www.flu.lanl.gov)

B/Vienna/1/99 wt gene coding for	Accession Nr. amino acid seq.	Accession Nr. nucleotide seq	Remarks
PB2, segment 1	ISDACH017	ISDNCHB017	in db listed as segment 2
PB1, segment 2	ISDACH016	ISDNCHB016	in db listed as segment 1
PA, segment 3	ISDACH015	ISDNCHB015	
HA, segment 4	ISDACH018	ISDNCHB018	
NP, segment 5	ISDACH013	ISDNCHB013	
NA, segment 6	ISDACH012	ISDNCHB012	
M, segment 7	ISDACH011	ISDNCHB011	
NS, segment 8	ISDACH014	ISDNCHB014	

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In addition, further passaging of strain B/Vienna/1/99 ca for 15 additional passages (i.e. a total of 37 passages on serum-free Vero cell culture) resulted in a mutant B/Vienna/1/99 ca37 (abbreviated BV37) with properties even superior to the ones of BV22. This mutant contains an increased number of mutations vis-à-vis BV22 and appears to be the currently most promising candidate for the production of a whole-virus vaccine, particularly for an attenuated influenza live vaccine, based on a non-recombinant influenza virus mutant. The additional mutations are listed in Table 13A below:

10 Table 13 A: Mutations for BV22 and BV37 compared to B/Vienna/1/99 wt 1st passage on Vero/SF

Segment (length in nucleotides)	Nucleotides changed				Protein (length in amino acids)	Amino acids changed			
	Pos.	BVie	BV22	BV37		Pos.	BVie	BV22	BV37
1 (2396)	-	-	-	-	PB2 (770)	-	-	-	-
2 (2369) (BV37: 2370)	594 2348	T -	<u>C</u> -	<u>C</u> <u>A</u>	PB1 (752)	-	-	-	-
3 (2305)	-	-	-	-	PA (726)	-	-	-	-
4 (1882)	457 1122 1299 1595	G C G G	A* C <u>T</u> A	A* <u>A</u> <u>G</u> A	HA ₀ (584)	142 363 422 521	A F K G	T* F <u>N</u> E	T* <u>L</u> K E
5 (1844)	128 212 330	C C T	<u>T</u> <u>C</u> C#	<u>T</u> <u>T</u> C#	NP (560)	23 51 -	S P -	F <u>P</u> -	F <u>L</u> -
6 (1557)	- 823 1135	- G T	- <u>A</u> C•	- G C•	NB (100) NA (466)	- 257 361	- R I	- <u>Q</u> T•	- R T•
7 (1190)	24 831 831 1029	G A A A	G <u>G</u> <u>G</u> A	<u>A</u> <u>G</u> <u>G</u> <u>G</u>	M1 (248) BM2 (109)	- 21 87	- M I	- <u>V</u> <u>I</u>	- <u>V</u> <u>V</u>
8 (1097)	116 -	G -	<u>A</u> -	<u>A</u> -	NS1 (281) NS2 (122)	25 -	A -	<u>T</u> -	<u>T</u> -

Comparison with influenza sequence database 13.2. 2001 (www.flu-lanl.gov):

a) unique mutations underlined in bold type;

b) mutations common with:

* B/Lee/40, B/Osaka/70, B/Kadoma/1076/99 (resulting amino acid: I)

+ B/Lee/40, B/Osaka/70

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- # often: B/Lee/40, B/Ann Arbor/1/66 ca & wt, B/Singapore/222/79, B/North Dakota/83, B/Norway/1/84, B/Ibaraki/2/85, B/Ann Arbor/1/86, B/Victoria/2/87, B/Aichi/5/88
- B/Kanagawa/73

- 5 It shall be understood that the influenza A and B master strains according to the present invention shall not be limited to the features and genetic characteristics explicitly listed in the tables herein but shall also comprise minor variations thereof as long as such variations are in the sense of the present invention and do not substantially alter any one of the functional features of the virus.
- 10 Such variations may occur, for instance, due to additional steps of virus multiplication or propagation (e.g. for the purpose of obtaining material for sequence analyses).
- Moreover, the gene sequences listed herein include the primer sequences (located at the beginning and at the end of each genome segment) that were
- 15 used along with the present invention, which primer sequences may differ from the corresponding true sequences of the viral genome segments of either or both the wildtype and the attenuated virus strains.

Example 5: Vaccine safety and efficacy

20

The subsequent data confirm temperature sensitivity and vaccine safety for influenza vaccines manufactured according to the present invention, e.g., as described in Example 1.

- 25 Table 14: Antibody response of mice after one intranasal immunisation without narcosis

Viruses	Number of responders ¹	GMT ³	Protection after challenge ²
PR8/Sing ca -2/6	0/6	< 4	5/6
PR8/Sing ca -ΔNS	4/6	6.7	5/6
PR8-wt	5/6	16.0	5/6

1 - number of animals with positive HI titer > 1:4

2 - number of animals without detectable virus in the lungs

3- Geometric mean titer of antibodies in serum

30

PR8wt – influenza strain A/PR/8/34 wildtype (H1N1), pathogenic for mice

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PR8/Sing ca-2/6 - is the reassortant between attenuated influenza strain

A/Sing/1/57 ca and PR8 wt, containing 2 genes (HA and NA) from PR8wt virus and all other genes from A/Sing/1/57 ca.

- PR8/Sing-ΔNS contains HA and NA genes from PR8wt, five genes from
 5 A/Sing/1/57 ca and the NS gene of PR8 origin lacking the NS1 coding sequence (NS1 deletion or knockout).

Table 15: Antibody response and protection of mice after intranasal immunisation with different variants of A/Singapore/1/57 virus (under narcosis)

Viruses	Responders ¹		GMT after two immunisations	Protection after challenge ⁴
	1-st immunisation	2-nd immunisation		
A/Sing/1/57/wt va ²	9/9	9/9	103.9	9/9
A/Sing/1/57/ca ³	8/10	10/10	55.7	8/10
A/Sing /57/ΔNS 87	1/10	10/10	27.9	8/10

¹ - number of animals with positive HI titer > 1:4

² - va- Vero-adapted

³ - ca - cold-adapted

⁴ - number of animals without detectable virus in the lungs

Table 16: Reproduction of wt, va and ca variants of A/Singapore/1/57 in mouse lungs^a

Viruses	Virus titer in mouse lungs post infection on day, PFU/ml ^b		
	2	4	6
A/Singapore/1/57/wt	1.6x10 ⁶	2.2x10 ⁵	1.4x10 ³
A/Singapore/1/57/wt va	2.5x10 ⁶	2.1x10 ⁶	1.0x10 ²
A/Singapore/1/57/ca	< 10	< 10	< 10

^a Mice were infected i.n. with 50 µl of virus fluid with a titer 1.0 x 10⁶ PFU/ml.

^b PFU/ml of 10% tissue suspension, titrated on MDCK cells.

Table 17: Virulence of wt and ca variants of A/Singapore/1/57 virus for ferrets

Viruses	Number of animals with fever post infection on day		
	1	2	3
A/Singapore/1/57 wt	FFF	NNN	NNN
A/Singapore/1/57 ca	NNN	NNN	NNN

Rectal temperature of animals was recorded twice a day and characterized as follows:

- 5 **N** - normal temperature from 38.1°C to 39.9 °C

F - fever, more than 40.0°C.

Each group consisted of 3 animals, which were immunized i.n. under ether narcosis with 1 ml of virus fluid with a titer of 2×10^6 PFU/ml.

- 10 Table 18: Reproduction of 2/6 reassortant of A/Hong Kong/1035/98 wt and A/Singapore/1/57/ca in mouse lungs^a

Viruses	Virus titer in mouse lungs on day 2-6 post infection, PFU/ml ^b		
	2	4	6
A/Hong Kong/1035/98 wt H1N1	6.8×10^4	2.0×10^4	< 10
A/Singapore/1/57/ca x A/Hong Kong/1035/98 wt	< 10	< 10	< 10

^a Mice were infected i.n. under ether narcosis with 50 µl of virus fluid.

- ^b PFU/ml of 10% tissue suspension, titrated on Vero/SF cells, data are given as mean value for 6 mice (the lungs of each animal were treated separately).

The reassortant contains the HA and NA genes from A/Hong Kong/1035/98 wt wildtype and the other 6 genes from A/Singapore/1/57/ca.

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Table 19: Virulence of 6/2 reassortant of A/Vienna/47/96 wt and
A/Singapore/1/57/ ca for ferrets

Viruses	Virus subtype	Number of animals with fever on day			
		1	2	3	Rhinitis ^a
<i>Master strain</i> A/Singapore/1/57/ ca	H2N2	NNN	NNN	NNN	<u>+</u>
<i>Epidemic virus</i> A/Vienna/47/96 wt	H3N2	NNN	FFF	FFF	+++
<i>Reassortant</i> A/Singapore/1/57/ca x Vienna/47/ 96 wt	H3N2	NNN	NNN	NNN	<u>+</u>

Animals were immunized i.n. under ether narcosis with 1 ml of virus, 2×10^8 PFU/ml.

5 N- normal temperature from 38.1°C to 39.9°C;

F- fever, more than 40.0°C.

^b +++ - severe rhinitis

± absence of rhinitis

- 10 The results presented in Tables 16 to 19 clearly demonstrate the safety of the vaccines containing the attenuated, temperature sensitive master strain or, in case of reassortants, of the vaccines based on the reassorted viruses composed of the "backbone" of the attenuated, temperature sensitive master strain (6 genes) and the HA and NA genes from, e.g., the pathogenic wildtype strain
- 15 A/Hong Kong/1035/98 wt.

Table 20: Ts and ca phenotype of B/Vienna/1/99

Virus	PFU/ml on Vero cells at	PFU/ml on MDCK cells at	
	25°C	33°C	39°C
B/Vienna/1/99 wt	< 300	4×10^6	4×10^5
B/Vienna/1/99 ca (BV22)	1×10^6	2.4×10^6	< 20

Table 21: Genetic stability of the ts phenotype of B/Vienna/1/99 ca

Virus	PFU/ml on MDCK cells	
	at	
	33°C	39°C
B/Vienna/1/99 wt	4×10^6	4×10^5
B/Vienna/1/99 ca (BV22)	2.4×10^6	< 20
B/Vienna/1/99 ca (BV22) after 5 passages at 33°C	8×10^5	< 20

The strain BV22 was passaged five times at high MOI on Vero cells. Then the ts-phenotype was controlled again. The strain remained temperature sensitive as can be seen in Table 21.

5

Table 22: Virulence of B/Vienna/1/99 ca and wt in mouse lungs

		PFU/ml* at day post infection		
Virus	organ	2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	1×10^2	1×10^2	20
B/Vienna/1/99 wt	lung	8×10^4	7×10^3	4.4×10^3
	nose	3.8×10^4	3.4×10^4	1.4×10^4

* 9 OF1 mice per strain were immunized intranasally under ether narcosis with 10^5 PFU. At the indicated days post infection 3 mice per group were sacrificed. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

10

The data show that moderate reproduction of the ca master strain candidate BV22 was possible in the nasal mucosa while the ts property of the virus prevented reproduction in the lungs.

15

Table 23: Ts and ca phenotype of the reassortant influenza B strain

Virus	PFU/ml on MDCK cells at	
	33°C	39°C
B/Vienna/1/99 wt	4×10^6	4×10^5
B/USSR/69 wt	1.6×10^6	4×10^4
B/Vienna/1/99 ca (BV22)	1.4×10^6	< 20
BV22 x B/USSR/69 (6/2)	8×10^6	< 20

A 6/2 reassortant strain containing HA and NA of the wild type influenza strain B/USSR/69 wt and the other 6 genome segments from B/Vienna/1/99 ca (BV22) was established. The origin of the hemagglutinin was tested by HI-assay, all other genome segments by RT-PCT and restriction analysis using methods known in the art.

Table 24: Virulence of the reassortant influenza B strain in mouse lungs

Virus	organ	PFU/ml* at day post infection		
		2	3	4
B/Vienna/1/99 ca (BV22)	lung	< 20	< 20	< 20
	nose	< 20	1×10^2	40
B/USSR/69 wt	lung	1.8×10^5	4×10^5	2.4×10^4
	nose	1.6×10^5	2×10^5	1.6×10^5
BV22 x B/USSR/69 wt (6/2)	lung	< 20	< 20	< 20
	nose	2.8×10^3	2×10^3	4×10^2

* 9 OF1 mice per strain were immunized intranasally under ether narcosis with 10^5 PFU. At the indicated days post infection 3 mice per group were sacrificed. Lungs and nasal turbinates were homogenized for a 10% (w/v) suspension in PBS def. A plaque assay of the suspensions was performed.

Example 6: Clinical study

The following vaccines (in the form of nasal sprays) were produced according to the present invention (e.g. as described in Example 1) for intranasal delivery. Composition per ml (after reconstitution of freeze-dried material):

- (1) Placebo: 2x SF-medium, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- (2) Vero-Vac H1: A/Beijing/262/95 (H1N1)-like preparation comprising 4.3×10^7 TCID₅₀ of 6/2 reassortant A/Singapore/1/57/ca with A/Hong Kong/1035/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- (3) Vero Vac H3: A/Sidney/5/97 (H3N2)-like preparation comprising 2.1×10^7 TCID₅₀ of 6/2 reassortant A/Singapore/1/57/ca with A/SW/7729/98; 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;
- (4) Russian trivalent vaccine (live influenza vaccine for adults):
A/17/Beijing/95/25 (H1N1) 1.1×10^8 EID₅₀

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- | | | |
|--|--------------------------|---------------------------------------|
| | A/17/Sidney/97/76 (H3N2) | 2.3x10 ⁷ EID ₅₀ |
| | B/60/Petersburg/95/20 | 1.1x10 ⁷ EID ₅₀ |
- (5) Monovalent Vero vaccine BV22: B/Beijing/184/93 - like preparation comprising 2x10⁶ TCID₅₀ of master strain candidate B/Vienna/1/99/ca (=BV22); 2x culture supernatant, 40mM HEPES buffer, 8% lactalbumin enzymatic hydrolysate, 4% trehalose;

The vaccines were administrated to 13 volunteers per each vaccination group. 550 µl of reconstituted vaccine (or placebo, respectively) were given intranasally to each patient on day 0 and for a second time on day 22 ± 1. The results are summarized in Table 25 below.

Safety results:

The total number of adverse events (AE) during five days after the first and second vaccination was 14 including 9 mild and 4 moderate AE. Only one volunteer showed severe AE, comprising an increase in body temperature up to 38.8°C within 3 hours after the first vaccination without any local or systemic symptoms. During the next four hours his temperature became normal again. After the first vaccination 7 AE were observed. One of them was local and six were systemic. After the second vaccination 2 local and 5 systemic AE were observed.

No significant difference in terms of safety was revealed between the groups of the study including the one with placebo. No serious AE related to the vaccination were observed except for the one mentioned above. Two of the moderate AE occurred in the H3N2 group (temperature elevation up to 37.6° and acute pharyngitis on day 3 in one volunteer; nasal obstruction, discomfort in the throat on day 22-24 and temperature elevation up to 37.5°C in another volunteer), and one in the H1N1 group (pain in the throat, rhinitis from day 22-26, temperature elevation up to 37 - 37.8°C between days 22-24).

Table 25: Response of seronegative volunteers to Vero Vac vaccines and to a trivalent Russian cold-adapted egg derived vaccine

No	Vaccine for immunization	Virus dose, TCID ₅₀ /ml or EID ₅₀ /ml	No. of volunteers	% of volunteers with at least 4-fold increase of serum HAI antibody titre to antigens		
				H1N1	H3N2	B
1	Placebo		13		(8)	
2	Vero Vac H1 (H1N1)	4.3x10 ⁷	13	38		
3	Vero Vac H3 (H3N2)	2.1x10 ⁷	13		67	
4	Russian trivalent vaccine: A/17/Beijing/95/25 H1N1 A/17/Sidney/97/76 H3N2 B/60/Petersburg/95/20	 1.1x10 ⁸ 2.3x10 ⁷ 1.1x10 ⁷	13	46	8	31
5	Vero vaccine BV22	2x10 ⁶	13			33

(8) patient developed spontaneous infection during course of study.

- 5 The results obtained from the clinical study thus confirm a very good safety of the vaccines produced according to the present invention and using the preferred influenza A and B master strain candidates of the present invention.

CLAIMS

We claim

1. A method for the manufacture of a whole-virus vaccine, preferably an attenuated live vaccine, comprising the steps of:
 - 5 a) infecting African Green Monkey Kidney (Vero) cells with a desired virus, wherein the Vero cells have been grown in and separated from a serum-free medium that is also free of non-serum proteins;
 - b) combining the infected cells with a suitable serum-free cell culture medium that is also free of non-serum proteins except for a protease and a
 - 10 nuclease; and
 - c) incubating the cells in the presence of said protease and said nuclease to allow for production of infectious virus and, simultaneously, for digestion of nucleic acid material released to the cell culture medium;
 - d) harvesting infectious virus by collecting virus-containing supernatant
 - 15 obtained from centrifugation of the cell culture; and
 - e) preparing a vaccine thereof comprising subjecting the virus-containing supernatant to at least one processing step selected from the group consisting of filtering, concentrating, freezing, freeze-drying, and stabilizing by addition of a stabilizing agent.
- 20 2. The method according to claim 1, which does not involve a step of protein separation or purification.
3. The method according to claim 1 or 2, which does not involve a step of
- 25 chromatographic separation or purification, and preferably does not contain any purification step other than centrifugation and/or filtration.
4. The method according to any one of claims 1 to 3, which comprises at least one step of sterile filtration of the virus-containing supernatant.
- 30 5. The method according to any one of claims 1 to 4, wherein the nuclease has DNase and/or RNase activity, and preferably is Benzonase.
6. The method according to any one of claims 1 to 5, wherein the protease
- 35 and the nuclease are added to the cell culture medium once prior to or at the beginning of incubation of the infected cells.

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7. The method according to any one of claims 1 to 6, wherein the protease comprises trypsin and/or trypsinogen of human recombinant or porcine origin which is present in the cell culture medium at an initial concentration of 0.5 - 10, preferably 2 - 5 µg per ml medium.
- 5 8. The method according to any one of claims 1 to 7, wherein the cell culture medium comprises nuclease at an initial concentration of 2 to 30, preferably 5 to 15, U per ml of medium.
- 10 9. The method according to any one of claims 1 to 8, wherein the incubation in step (a) is carried out for 10 to 120 minutes, preferably for 30 to 60 minutes.
10. The method according to any one of claims 1 to 9, wherein the virus is
15 selected from the group consisting of a wildtype virus, a primary isolate directly obtained from an infected individual, a recombinant virus, an attenuated virus, a Vero adapted virus, a cold-adapted virus, a temperature-sensitive virus, and a reassortant virus.
- 20 11. The method according to any one of claims 1 to 10, wherein the virus is an influenza A virus, preferably of subtype H3N2 or H1N1, or an influenza B virus.
12. The method according to any one of claims 1 to 11, wherein the virus
25 has an interferon inducing and/or interferon sensitive phenotype.
13. The method according to any one of claims 1 to 12, wherein the virus is an influenza virus selected from the group consisting of strains
A/Sing/1/57ca, A/Sing/1/57ca/ΔNS 87, A/Sing/1/57ca/ΔNSPR8,
30 A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, B/Vienna/99/ca37 and any attenuated variants and reassortants derived from any one of these strains.
14. A whole-virus vaccine, preferably an attenuated live vaccine, characterized in that in its ready-for-use form it comprises essentially
35 unmodified, optionally filtered and/or concentrated, virus-containing supernatant of a serum-free and protein-free Vero cell culture used for production of said virus.

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15. The vaccine according to claim 14, characterized in that it selectively agglutinates human erythrocytes but not chicken erythrocytes.

16. The vaccine according to claim 14 or 15, characterized in that it contains
5 a suitable stabilizing agent.

17. The vaccine according to any one of claims 14 to 16, characterized in that it is in the form of a liquid, frozen or freeze-dried preparation, optionally suitable for intranasal delivery.

10

18. The vaccine according to any one of claims 14 to 17, characterized in that it is a live attenuated vaccine, preferably comprising whole influenza virus.

19. The vaccine according to any one of claims 14 to 18, characterized in
15 that it comprises at least one influenza virus having a phenotype with one or more characteristics selected from the group consisting of cold adapted, temperature sensitive, interferon inducing, interferon sensitive.

20. The vaccine according to claim 18, wherein the influenza virus is
20 selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ Δ NS 87, A/Sing/1/57ca/ Δ NSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains.

25 21. The vaccine according to claim 14, obtainable by a method of manufacture as defined in any one of claims 1 to 13.

22. A whole-virus vaccine, preferably an attenuated live vaccine, comprising at least one influenza virus selected from the group consisting of strains
30 A/Sing/1/57ca, A/Sing/1/57ca/ Δ NS 87, A/Sing/1/57ca/ Δ NSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and reassortants derived from any one of these strains.

23. The vaccine according to claim 21, characterized in that it selectively
35 agglutinates human erythrocytes but not chicken erythrocytes.

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24. The vaccine according to claim 22 or 23, obtainable by a method of manufacture according to any one of claims 1 to 13.

25. Use of a vaccine defined in any one of claims 14 to 24 for prophylactic
5 or therapeutic administration against viral infection.

26. Use of at least one influenza virus selected from the group consisting of strains A/Sing/1/57ca, A/Sing/1/57ca/ Δ NS 87, A/Sing/1/57ca/ Δ NSPR8, A/Sing/1/57ca/NS124PR8, B/Vienna/1/99ca, and any attenuated variants and
10 reassortants derived from any one of these strains, for the manufacture of a vaccine, preferably for the manufacture of a live attenuated influenza vaccine.

1/1

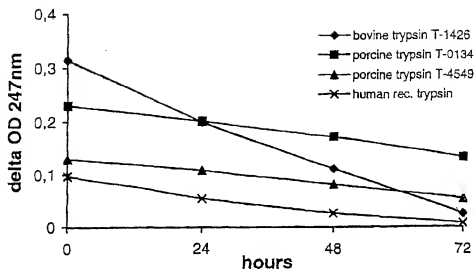


Fig. 1

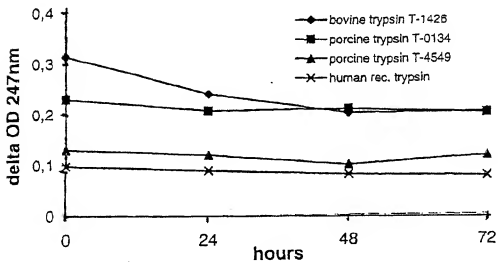


Fig. 2

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Katinger, Dietmar

Romanova, Julia

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 agggaaactcg tcctttatga caaagaagaa ataaggcgaa tctggcgcca agctaataat 420
 ggtgatgatg caacagctgg tctgactcac atgatgatct ggcattccaa tttgaatgat 480
 acacaatacc agaggacaag agctcttggt cgcaccggaa tggatccag gatgtgctct 540
 ttgatgcagc gttcgactct ccctaggagg tctggagcgc caggcgctgc agtcaaagga 600
 gttgggacaa tgggtgatga gttgatcagg atgatcaaac gtgggatcaa tgatcggaac 660
 ttctggagag gtgagaatgg gcggaataca aggattgctt atgagagaat gtgcaacatt 720
 ctcaaaggaa aatttcaaac agctgcacaa agagcaatga tggatcaagt gagagaagc 780
 cggaacccag gaaatgtgga gatcgaagat ctcatcttct tggcacggtc tgcactcata 840
 ttgagagggt cagttgctca caaatcttgt ctgcctgctc gtgtgtatgg aactgccgta 900
 gccagtggtg acgacttoga aaaagaggga tactctttag tagggataga ccctttcaaa 960
 ctgcttcaaa acagccaagt atacagccta atcagacoga acgagaatcc agcacacaag 1020
 agtcaagctg tgtggatggo atgcaattct gctgcatttg aagatctaaq agtatcaagc 1080
 ttcatcagag ggaccaaaat aatcccaagg gggaaacttt ccactagagg agtacaattt 1140
 gcttcaaatg aaaacatgga tactatggaa tcaagtactc ttgaacttag aagcaggtac 1200
 tgggcccataa ggaccagaag tggagggaac actaatcaac agagggcctc tgcaggtcaa 1260
 atcagtgtag aacctacgtt ttctgtgcaa agaaaacctc catttgacaa aacaacctatc 1320
 atggcagcat tcaactggaa tgcagaggga agaacaatcag acatgagggc agaaatcata 1380
 aggatgatgg aaggtgcaca accagaagaa gtgtccttcc agggcggggg agtcttgag 1440
 ctctcgagc aaaagccaac gaaccgacgt gtgcctctct ttgacatgag taatgaagga 1500
 tctattttct tcggagacaa tgcagaggag tacgacaatt aaggaaaaat acccttggtt 1560
 ctact 1565

<210> 6

<211> 1466

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 6

agcaaaagca ggagtgaaga tgaatccaaa tcaaaaagata ataacaattg gctctgtctc 60
 tctcaccatt gcaacagtat gcttctcat gcagattgcc atcctggcaa ctactgtgac 120
 attgcatttt aaacaacatg agtgcgctc ccccgcgagc aaccaagtaa tgccatgtga 180
 accaataata atagaagga acataacaga gatagtgtat ttgaataaca ccacataga 240

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gaaagagatt  tgccccgaag  tagtgggaata  cagaaattgg  tcaaagccgc  aatgtcaaat  300
tacaggatttt  gcaccttttt  ctaaggacaa  ttcaatccgg  ctttctgctg  gtggggacat  360
ttgggtgcagc  agagaacott  atgtgtcatg  cgatcctggc  aagtgttatc  aatttgcact  420
cgggcagggg  accacactat  acaacaaaca  ttcaaatggc  acaatacatg  atagaatccc  480
tcacatgaacc  ctattaatga  atgagttggg  tgttccattt  catttaggaa  ccaaacaagt  540
gtgtgtagca  tggctccagc  caagttgtca  cgatggaaaa  gcattggtgc  atgttttgtt  600
cactggggat  gatagaaatg  cgactgctag  cttcatttat  gacgggagcg  ttgtggacag  660
tatttggttca  tggctctcaa  atatcctcag  gaccaggag  tcggaaatgcg  ttgtgtatcaa  720
tgggactctgc  acagtagtaa  tgactgatgg  aagtgcatac  ggaagagccg  atactagaat  780
actattcaatt  aaagagggga  aaattgtccg  tattagccca  ttgtcaggaa  gtgtccagca  840
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ctggaaaggg  tctaataagg  cgttataga  cataaatatg  gaagattata  gcattgatct  960
cagttattgt  tgctcagggc  ttgttggcga  cacaccagg  aacgacgaca  gctctagcaa  1020
tgacaattgc  agggatccca  acaatgagag  agggaatcca  ggaagtgaag  gctgggcttt  1080
tgacaatgga  gatgatgtat  ggaagggaag  aacaatcaac  aaagattcac  gctcagggtta  1140
tgaaactttc  aaagtcatgt  gtggttggtc  cacacctaat  tccaaatcgc  aggtcaaatg  1200
acaggtcata  gttgacaaca  ataattgttc  tggttactct  ggtattttct  ctgttgaggg  1260
caaaagctgc  atcaataggt  gcttttatgt  ggagttgata  aggggaaggg  cacaggagac  1320
tagagtattg  tggacctcaa  acagtattgt  tgtgttttgt  ggcacttcag  gtacttatgg  1380
aaacggctca  tggcctgatg  gggcgaaacat  caatttcgat  cctatataag  ctttcgcgat  1440
tttagaaaaa  actccttggt  tctact  1466

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<210> 7

<211> 1027

<212> DNA

<213> Influenza virus A/Singapore/1/57/ca

<400> 7

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agcaaaagca  ggtagatatt  gaaagatgag  tcttctaacc  gaggtcgaaa  cgtatgttct  60
ctctatgtgc  ccgtcaggcc  cctccaaagc  cgagatcgca  cagagacttg  aagatgtctt  120
tgctgggaag  aacaccgato  ttgaggctct  catggaatgg  ctaagacaaa  gaaccaatct  180
gtcacctctg  actaaggggg  ttttgggatt  tgtattccag  ctccagctgc  ccagtgagcg  240
aggactcgag  cgtagacgct  ttgtccaaaa  tgccctcaat  ggggaatggg  atccaaataa  300
catggacaga  gcagttaaac  tgtataaaaa  gcttaagagg  gagataacat  tccattgggc  360
caaaagaata  gcgctcagtt  attctgctgg  tgcacttgcc  agttgtatgg  gcctcatata  420
caacaggatg  ggggctgtga  ccactgaagt  ggcctttggc  ctggatgtgt  caacctgtga  480
acagattgct  gactcccaco  ataggttcca  taggcaaatg  gtgacaacca  ccaatccaat  540
aatgaagaca  gagaacagaa  tggttctggc  cagcaactac  gctaaaggcta  tggagcaaat  600
ggctggatcg  agtgagcaag  cagcagaggg  catggaggtt  gctagtccag  ccaggcaaat  660
gggtcaggca  atgagagcca  ttgggaactca  tctctagctcc  agtgcctggc  taaaagatga  720
tctctttgaa  aatttgcagg  cctatocaaa  acgaatgggg  gtgcagatgc  aacgattcaa  780
gtgacctctc  tgttgttgcc  gcgagtatca  ttgggatctt  gcacttgata  ttgtggattc  840
ttgatgctct  ttttttcaaa  tgcattttatc  gcttctttaa  acacggtctg  aaaagagggc  900
cttctacgga  aggagatcca  gagtctatga  gggaagaata  tcgaaaggaa  cagcagatgc  960
ctgtggatgc  tgaogatagt  cattttgtca  gcataagagct  ggagtaaaaa  actaccttgt  1020
tctact  1027

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<210> 8

<211> 890
 <212> DNA
 <213> Influenza virus A/Singapore/1/57/ca

<400> 8
 agcaaaagca gggtagacaaa gacataatgg atcctaacac tgtgtcaagc ttccaggtag 60
 attgtcttct ttggcatgto cgcaaacag ttgcagacca agaactaggt gatgcccac 120
 tctttgatgc gcttcgcccga gatcagaagt ccttaagggg aagaggcagc actctcggtc 180
 tgaactcga aacagccacc cgtgttgaa agcagatagt ggagaggatt ctgaagggaag 240
 aatccgatga ggcacttaaa atgaccatgg cctccgcacc tgccttcgca tacctaactg 300
 acatgactat tgaggaaatg tcaagggact ggttcattgt aatgcccaag cagaaagtgt 360
 caggccctct ttgtatcaga atggaccagg caatcatgga taagaacatc atattgaaag 420
 cgaatttcag tgtgattttt gaccggctag agaccctaatt attactaagg gctttcaccg 480
 aagagggagc aattgttggc gaaatttcac cattgccttc tcttcacgga cataactaag 540
 aggatgtcaa aaatgcaatt ggggtcctca tcggaggact tgaatggaat gataacacag 600
 ttgagttctc taaaactcta cagagattcg cttggagaaa cagtaagtga aatgggagac 660
 ctccactcac tccaaaacag aaacggaaaa tggcgagaac aattaggtca aaagttcgaa 720
 gaaataagat ggctgattga agaagtgaga cacaattga agataacaga gaatagtttt 780
 gagcaataa catttatgca agccttacag ctgctatttg aagtggaca agagataaga 840
 actttctcgt ttcagcttat ttaatgataa aaacaccct tgtttctact 890

<210> 9
 <211> 771
 <212> PRT
 <213> Influenza virus A/Singapore/1/57/ca

<400> 9
 Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr
 1 5 10 15
 Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys
 20 25 30
 Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys
 35 40 45
 Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr
 50 55 60
 Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
 65 70 75 80
 Met Asn Asp Ala Gly Ser Asp Arg Val Met Val Ser Pro Leu Ala Val
 85 90 95
 Thr Trp Trp Asn Arg Asn Gly Pro Met Thr Ser Thr Val His Tyr Pro
 100 105 110

Lys Ile Tyr Lys Thr Tyr Phe Glu Lys Val Glu Arg Leu Lys His Gly
 115 120 125
 Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg
 130 135 140
 Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln
 145 150 155 160
 Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile
 165 170 175
 Leu Thr Ser Glu Ser Gln Leu Thr Thr Thr Lys Glu Lys Lys Glu Glu
 180 185 190
 Leu Gln Asp Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu
 195 200 205
 Arg Glu Leu Val Arg Lys Thr Arg Phe Leu Pro Val Ala Gly Gly Thr
 210 215 220
 Ser Ser Val Tyr Ile Glu Val Leu His Leu Thr Gln Gly Thr Cys Trp
 225 230 235 240
 Glu Gln Met Tyr Thr Pro Gly Gly Glu Val Arg Asn Asp Asp Val Asp
 245 250 255
 Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Ala Val
 260 265 270
 Ser Ala Asp Pro Leu Ala Ser Leu Leu Glu Met Cys His Ser Thr Gln
 275 280 285
 Ile Gly Gly Thr Arg Met Val Asp Ile Leu Arg Gln Asn Pro Thr Glu
 290 295 300
 Glu Gln Ala Val Asp Ile Cys Lys Ala Ala Met Gly Leu Arg Ile Ser
 305 310 315 320
 Ser Ser Phe Ser Phe Gly Gly Phe Thr Phe Lys Arg Thr Ser Gly Ser
 325 330 335
 Ser Val Lys Ile Glu Glu Glu Val Leu Thr Gly Asn Leu Gln Thr Leu
 340 345 350
 Lys Ile Arg Val His Glu Gly Tyr Glu Glu Phe Thr Met Val Gly Lys
 355 360 365

Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Ile Gln Leu
 370 375 380

Ile Val Ser Gly Arg Asp Glu Gln Ser Ile Ala Glu Ala Ile Ile Val
 385 390 395 400

Ala Met Val Phe Ser Gln Glu Asp Cys Met Ile Lys Ala Val Arg Gly
 405 410 415

Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His
 420 425 430

Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Gln Asn
 435 440 445

Trp Gly Ile Glu His Ile Asp Asn Val Met Gly Met Ile Gly Val Leu
 450 455 460

Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Val Arg Val
 465 470 475 480

Ser Lys Met Gly Val Asp Glu Tyr Ser Ser Ala Glu Arg Val Val Val
 485 490 495

Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu
 500 505 510

Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr
 515 520 525

Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser
 530 535 540

Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val
 545 550 555 560

Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu
 565 570 575

Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr
 580 585 590

Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly
 595 600 605

Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala
 610 615 620

Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val
625 630 635 640

Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Val Phe
645 650 655

Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala
660 665 670

Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ser Gly Val Glu Ser
675 680 685

Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr
690 695 700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu
705 710 715 720

Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys
725 730 735

Arg Lys Arg Asp Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys
740 745 750

Arg Ile Arg Met Ala Ile Asn Xaa Cys Xaa Ile Val Xaa Lys Arg Pro
755 760 765

Cys Phe Tyr
770

<210> 10

<211> 757

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 10

Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn
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Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His
20 25 30

Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln
35 40 45

Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala Pro
50 55 60

Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
 65 70 75 80
 Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu
 85 90 95
 Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Leu Glu Thr Met Glu
 100 105 110
 Val Ile Gln Gln Thr Arg Val Asp Lys Leu Thr Gln Gly Arg Gln Thr
 115 120 125
 Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala
 130 135 140
 Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser
 145 150 155 160
 Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Ile Glu Ser Met Asp Lys
 165 170 175
 Glu Glu Met Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg
 180 185 190
 Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys
 195 200 205
 Lys Gln Arg Leu Asn Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu
 210 215 220
 Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
 225 230 235 240
 Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu
 245 250 255
 Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro
 260 265 270
 Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys
 275 280 285
 Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly
 290 295 300
 Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala
 305 310 315 320

Met Ile Thr Tyr Ile Thr Arg Asn Gln Pro Glu Trp Phe Arg Asn Val
325 330 335

Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly
340 345 350

Lys Gly Tyr Met Phe Glu Ser Lys Ser Met Lys Leu Arg Thr Gln Ile
355 360 365

Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser
370 375 380

Thr Arg Lys Lys Ile Glu Lys Ile Arg Pro Leu Leu Ile Asp Gly Thr
385 390 395 400

Val Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser
405 410 415

Thr Val Ile Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr
420 425 430

Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala
435 440 445

Leu Ile Val Asn Ala Pro Asn His Glu Gly Ile Gln Ala Gly Val Asp
450 455 460

Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys
465 470 475 480

Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe
485 490 495

Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe
500 505 510

Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr
515 520 525

Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala
530 535 540

Gln Met Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg
545 550 555 560

Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu
565 570 575

Lys Lys Leu Trp Glu Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser
 580 585 590
 Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu
 595 600 605
 Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu
 610 615 620
 Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val
 625 630 635 640
 Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu
 645 650 655
 Tyr Asp Ala Val Ala Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg
 660 665 670
 Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met
 675 680 685
 Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser
 690 695 700
 Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser
 705 710 715 720
 Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys
 725 730 735
 Lys Glu Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu
 740 745 750
 Leu Arg Arg Gln Lys
 755

<210> 11
 <211> 716
 <212> PRT
 <213> Influenza virus A/Singapore/1/57/ca

<400> 11
 Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
 1 5 10 15
 Ala Glu Arg Ala Met Lys Glu Tyr Gly Glu Asp Leu Lys Ile Glu Thr

20					25					30						
Asn	Lys	Phe	Ala	Ala	Ile	Cys	Thr	His	Leu	Glu	Val	Cys	Phe	Met	Tyr	
35					40					45						
Ser	Asp	Phe	His	Phe	Ile	Asn	Glu	Gln	Gly	Glu	Ser	Ile	Ile	Val	Glu	
50					55					60						
Leu	Asp	Asp	Pro	Asn	Ala	Leu	Leu	Lys	His	Arg	Phe	Glu	Ile	Ile	Glu	
65					70					75					80	
Gly	Arg	Asp	Arg	Thr	Met	Ala	Trp	Thr	Val	Val	Asn	Ser	Ile	Cys	Asn	
85					90					95						
Thr	Thr	Gly	Ala	Glu	Lys	Pro	Lys	Phe	Leu	Pro	Asp	Leu	Tyr	Asp	Tyr	
100					105					110						
Lys	Glu	Asn	Arg	Phe	Ile	Glu	Ile	Gly	Val	Thr	Arg	Arg	Glu	Val	His	
115					120					125						
Ile	Tyr	Tyr	Leu	Glu	Lys	Ala	Asn	Lys	Ile	Lys	Ser	Glu	Lys	Thr	His	
130					135					140						
Ile	His	Ile	Phe	Ser	Phe	Thr	Gly	Glu	Glu	Met	Ala	Thr	Lys	Ala	Asp	
145					150					155					160	
Tyr	Thr	Leu	Asp	Glu	Glu	Ser	Arg	Ala	Arg	Ile	Lys	Thr	Arg	Leu	Phe	
165					170					175						
Thr	Ile	Arg	Gln	Glu	Met	Ala	Ser	Arg	Gly	Leu	Trp	Asp	Ser	Phe	Arg	
180					185					190						
Gln	Ser	Glu	Arg	Gly	Glu	Glu	Thr	Ile	Glu	Glu	Arg	Phe	Glu	Ile	Thr	
195					200					205						
Gly	Thr	Met	Arg	Arg	Leu	Ala	Asp	Gln	Ser	Leu	Pro	Pro	Asn	Phe	Ser	
210					215					220						
Cys	Leu	Glu	Ile	Phe	Arg	Ala	Tyr	Val	Asp	Gly	Phe	Glu	Pro	Asn	Gly	
225					230					235					240	
Tyr	Ile	Glu	Gly	Lys	Leu	Ser	Gln	Met	Ser	Lys	Glu	Val	Asn	Ala	Lys	
245					250					255						
Ile	Glu	Pro	Phe	Leu	Lys	Thr	Thr	Pro	Arg	Pro	Ile	Arg	Leu	Pro	Asp	
260					265					270						
Gly	Pro	Pro	Cys	Ser	Gln	Arg	Ser	Lys	Phe	Leu	Leu	Met	Asp	Ala	Leu	

275	280	285
Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu		
290	295	300
Tyr Asp Ala Ile Lys Cys Met Arg Thr Phe Phe Gly Trp Lys Glu Pro		
305	310	315 320
Tyr Val Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Leu		
325	330	335
Ser Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu		
340	345	350
Lys Ile Pro Arg Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp		
355	360	365
Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys		
370	375	380
Arg Asp Ile Ser Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu		
385	390	395 400
Arg Ser Leu Ser Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu		
405	410	415
Leu Thr Asn Ser Ile Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val		
420	425	430
Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala		
435	440	445
Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr		
450	455	460
Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe		
465	470	475 480
Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg		
485	490	495
Lys Thr Asn Leu Tyr Gly Phe Ile Val Lys Gly Arg Ser His Leu Arg		
500	505	510
Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr		
515	520	525
Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu		

530	535	540
Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Val Ser Arg Pro		
545	550	555 560
Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys		
565	570	575
Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile		
580	585	590
Glu Ser Met Ile Glu Ala Gln Ser Ser Val Lys Glu Lys Asp Met Thr		
595	600	605
Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser		
610	615	620
Pro Lys Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu		
625	630	635 640
Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu		
645	650	655
Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Val Val Gln Ala Leu		
660	665	670
Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu		
675	680	685
Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala		
690	695	700
Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Arg		
705	710	715

<210> 12

<211> 562

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 12

Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Asp
1 5 10 15

Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Lys Val Asp
20 25 30

Thr Ile Leu Glu Gln Asn Val Thr Val Thr His Ala Lys Asp Ile Leu
 35 40 45

Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro
 50 55 60

Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro
 65 70 75 80

Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu
 85 90 95

Lys Glu Asn Pro Arg Asp Gly Leu Cys Tyr Pro Gly Ser Phe Asn Asp
 100 105 110

Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys
 115 120 125

Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly
 130 135 140

Gly Ser Arg Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn
 145 150 155 160

Met Val Trp Leu Thr Lys Lys Glu Ser Asn Tyr Pro Val Ala Lys Gly
 165 170 175

Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val
 180 185 190

His His Pro Asn Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val
 195 200 205

Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr
 210 215 220

Pro Asp Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met
 225 230 235 240

Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu
 245 250 255

Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys
 260 265 270

Arg Gly Asn Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys
 275 280 285

Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro
 290 295 300

Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val
 305 310 315 320

Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Pro Arg Asn Val Pro Gln
 325 330 335

Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly
 340 345 350

Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn
 355 360 365

Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala
 370 375 380

Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Met Asn
 385 390 395 400

Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg
 405 410 415

Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp
 420 425 430

Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu
 435 440 445

Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met
 450 455 460

Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe
 465 470 475 480

Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr
 485 490 495

Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu
 500 505 510

Ile Lys Gly Val Lys Leu Ser Ser Met Gly Val Tyr Gln Ile Leu Ala
 515 520 525

Ile Tyr Ala Thr Val Ala Gly Ser Leu Ser Leu Ala Ile Met Met Ala
 530 535 540

Gly Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu Gln Cys Arg Ile
 545 550 555 560

Cys Ile

<210> 13

<211> 506

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 13

Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
 1 5 10 15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met
 20 25 30

Ile Asp Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
 35 40 45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
 50 55 60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
 65 70 75 80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
 85 90 95

Tyr Lys Arg Val Asn Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp
 100 105 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp
 115 120 125

Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn
 130 135 140

Asp Thr Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
 145 150 155 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
 165 170 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu
 180 185 190

Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
 195 200 205
 Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
 210 215 220
 Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Met Asp
 225 230 235 240
 Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
 245 250 255
 Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
 260 265 270
 Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Thr Ala Val Ala Ser Gly
 275 280 285
 Tyr Asp Phe Glu Lys Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
 290 295 300
 Lys Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu
 305 310 315 320
 Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys Asn Ser Ala
 325 330 335
 Ala Phe Glu Asp Leu Arg Val Ser Ser Phe Ile Arg Gly Thr Lys Val
 340 345 350
 Ile Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
 355 360 365
 Glu Asn Met Asp Thr Met Glu Ser Ser Thr Leu Glu Leu Arg Ser Arg
 370 375 380
 Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg
 385 390 395 400
 Ala Ser Ala Gly Gln Ile Ser Val Gln Pro Thr Phe Ser Val Gln Arg
 405 410 415
 Asn Leu Pro Phe Asp Lys Thr Thr Ile Met Ala Ala Phe Thr Gly Asn
 420 425 430
 Ala Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met
 435 440 445

Glu Gly Ala Lys Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe
 450 455 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
 465 470 475 480

Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
 485 490 495

Asp Asn Xaa Gly Lys Ile Pro Leu Phe Leu
 500 505

<210> 14

<211> 469

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 14

Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Val Ser Leu Thr
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Ile Ala Thr Val Cys Phe Leu Met Gln Ile Ala Ile Leu Ala Thr Thr
 20 25 30

Val Thr Leu His Phe Lys Gln His Glu Cys Asp Ser Pro Ala Ser Asn
 35 40 45

Gln Val Met Pro Cys Glu Pro Ile Ile Ile Glu Arg Asn Ile Thr Glu
 50 55 60

Ile Val Tyr Leu Asn Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Glu
 65 70 75 80

Val Val Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Gln Ile Thr Gly
 85 90 95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
 100 105 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Gly Lys
 115 120 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Tyr Asn Lys His
 130 135 140

Ser Asn Gly Thr Ile His Asp Arg Ile Pro His Arg Thr Leu Leu Met

145	150	155	160
Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Val			
165		170	175
Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val			
180	185		190
Cys Val Thr Gly Asp Asp Arg Asn Ala Thr Ala Ser Phe Ile Tyr Asp			
195	200	205	
Gly Arg Leu Val Asp Ser Ile Gly Ser Trp Ser Gln Asn Ile Leu Arg			
210	215	220	
Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val			
225	230	235	240
Met Thr Asp Gly Ser Ala Ser Gly Arg Ala Asp Thr Arg Ile Leu Phe			
245	250		255
Ile Lys Glu Gly Lys Ile Val Arg Ile Ser Pro Leu Ser Gly Ser Ala			
260	265		270
Gln His Ile Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Pro Asp Val Arg			
275	280		285
Cys Ile Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Val Ile Asp			
290	295	300	
Ile Asn Met Glu Asp Tyr Ser Ile Asp Ser Ser Tyr Val Cys Ser Gly			
305	310	315	320
Leu Val Gly Asp Thr Pro Arg Asn Asp Asp Ser Ser Ser Asn Ser Asn			
325	330		335
Cys Arg Asp Pro Asn Asn Glu Arg Gly Asn Pro Gly Val Lys Gly Trp			
340	345		350
Ala Phe Asp Asn Gly Asp Asp Val Trp Met Gly Arg Thr Ile Asn Lys			
355	360		365
Asp Ser Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Gly Gly Trp Ser			
370	375	380	
Thr Pro Asn Ser Lys Ser Gln Val Asn Arg Gln Val Ile Val Asp Asn			
385	390	395	400
Asn Asn Trp Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser			

405

410

415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Pro Gln
 420 425 430

Glu Thr Arg Val Trp Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
 435 440 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asn Ile
 450 455 460

Asn Phe Met Pro Ile
 465

<210> 15

<211> 252

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 15

Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro
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Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
 20 25 30

Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr
 35 40 45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
 50 55 60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
 65 70 75 80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Arg Ala
 85 90 95

Val Lys Leu Tyr Lys Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
 100 105 110

Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
 115 120 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
 130 135 140

Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser His His Arg
 145 150 155 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
 165 170 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
 180 185 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
 195 200 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser
 210 215 220

Ser Ser Ala Gly Leu Lys Asp Asp Leu Leu Glu Asn Leu Gln Ala Tyr
 225 230 235 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
 245 250

<210> 16

<211> 97

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 16

Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly
 1 5 10 15

Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Ser Ile
 20 25 30

Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp Arg Leu Phe Phe
 35 40 45

Lys Cys Ile Tyr Arg Phe Phe Lys His Gly Leu Lys Arg Gly Pro Ser
 50 55 60

Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln
 65 70 75 80

Gln Ser Ala Val Asp Ala Asp Asp Ser His Phe Val Ser Ile Glu Leu
 85 90 95

Glu

<210> 17

<211> 237

<212> PRT

<213> Influenza virus A/Singapore/1/57/ca

<400> 17

Met Asp Pro Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp
 1 5 10 15

His Val Arg Lys Gln Val Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe
 20 25 30

Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser
 35 40 45

Thr Leu Gly Leu Asn Ile Glu Thr Ala Thr Arg Val Gly Lys Gln Ile
 50 55 60

Val Glu Arg Ile Leu Lys Glu Glu Ser Asp Glu Ala Leu Lys Met Thr
 65 70 75 80

Met Ala Ser Ala Pro Ala Ser Arg Tyr Leu Thr Asp Met Thr Ile Glu
 85 90 95

Glu Met Ser Arg Asp Trp Phe Met Leu Met Pro Lys Gln Lys Val Ser
 100 105 110

Gly Pro Leu Cys Ile Arg Met Asp Gln Ala Ile Met Asp Lys Asn Ile
 115 120 125

Ile Leu Lys Ala Asn Phe Ser Val Ile Phe Asp Arg Leu Glu Thr Leu
 130 135 140

Ile Leu Leu Arg Ala Phe Thr Glu Glu Gly Ala Ile Val Gly Glu Ile
 145 150 155 160

Ser Pro Leu Pro Ser Leu Pro Gly His Thr Asn Glu Asp Val Lys Asn
 165 170 175

Ala Ile Gly Val Leu Ile Gly Gly Leu Glu Trp Asn Asp Asn Thr Val
 180 185 190

Arg Val Ser Lys Thr Leu Gln Arg Phe Ala Trp Arg Asn Ser Asn Glu
 195 200 205

Asn Gly Arg Pro Pro Leu Thr Pro Lys Gln Lys Arg Lys Met Ala Arg

210 215 220

Thr Ile Arg Ser Lys Val Arg Arg Asn Lys Met Ala Asp
 225 230 235

<210> 18
 <211> 121
 <212> PRT
 <213> Influenza virus A/Singapore/1/57/ca

<400> 18
 Met Asp Pro Asn Thr Val Ser Ser Phe Gln Asp Ile Leu Met Arg Met
 1 5 10 15
 Ser Lys Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile
 20 25 30
 Thr Gln Phe Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Thr
 35 40 45
 Val Met Arg Met Gly Asp Leu His Ser Leu Gln Asn Arg Asn Gly Lys
 50 55 60
 Trp Arg Glu Gln Leu Gly Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile
 65 70 75 80
 Glu Glu Val Arg His Lys Leu Lys Ile Thr Glu Asn Ser Phe Glu Gln
 85 90 95
 Ile Thr Phe Met Gln Ala Leu Gln Leu Leu Phe Glu Val Glu Gln Glu
 100 105 110
 Ile Arg Thr Phe Ser Phe Gln Leu Ile
 115 120

<210> 19
 <211> 2396
 <212> DNA
 <213> Influenza B/Vienna/1/99/ca

<400> 19
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 taagaaaatt caatacatca agaattgaaa agaacccttc attaaggatg aagtgggcaa 180
 tgtgttctaa ttttcccttg gctttgacca agggtgacat ggcaaacaga atcccccttg 240
 aatacaaggg aatacaactt aaacaaatg ctgaagacat aggaaccaaa ggccaaatgt 300


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gctcaatagc agcagttacc tgggtggaata catatggacc aataggagat actgaaggtt 360
tcgaaaaagt ctacgaaagc ttttttctca gaagatgag acttgacaat gccacttggg 420
gccgaataac ttttggccca gttgaaagag taagaaaaag ggtactgcta aacctctcca 480
ccaagcaaat gcctccagat gaagcaagta atgtgataat ggaaatatgt ttccctaagg 540
aagcaggaat accaagagaa tctacttggg tacataggga actgataaaa gaaaaaagag 600
aaaaattgaa aggaacgatg ataactccca ttgtactggc atacatgctt gagagggaat 660
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gaatgatgtc attaaaaggg aaaattgaag atgaagaag gaatagatca atgggggaatg 2100
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gaaagccogt taaagtgtt aaaaggaaaa gatatagtgc tttatccaat gacatttccac 2280
aaggaaatga gagacaaaga atgacagttg agtccatggg gtgggacctg agctaataata 2340
aatttatcca ttaattcaat gaatgcaatt gagtgaaaaa tgctcgtgtt tctcat 2396

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<210> 20

<211> 2369

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 20

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agcagaagcg gagcctttaa gatgaatata aatccttatt ttctcttcat agatgtaccc 60
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acgggaacag gccacacaat agacaccogt atcagaaacac atgagtactc gaacaaggga 180
aaacagtatg tttctgacat cacaggatgt acaatggtag atccaacaaa tggaccatta 240
cccgaaagca atgagccaaag tgcctatgca caattagatt gcgttctgga ggccttggat 300
agaatggatg aggaacatcc aggtctgttt caagcagcctc cacagaatgc catggaggca 360

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ctaattggtca caactgtaga caaattaacc caggggagac agactttcga ttggacagta 420
tgccagaaatc agcctgctgc aacggcacta aacacacaa taacctctct taggttgaat 480
gattttgaatg gagctgacaa ggggtggattg gtaccttttt gccaaagatat catttgattca 540
ttagacaagc ctgaaatgac ttctttctca gtaaagaata taaagaaaaa attccctgct 600
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gtccgaaga gtccatggg gttattggtc atcattggat acatgtgata aacaaatgat 2340
taaaatgaaa aaaggctcgt gtttctact 2369

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<210> 21

<211> 2305

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 21

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aaccagcaat gctattcaac atctgogtcc atctagagggt ttgctatgta ataagtga 180
tgaattttct tgacgaagaa ggaagagcat atacagcatt agaaggcaaa gggaagaa 240
aaaatttgag accacaatat gaagtaattg agggaatgcc aagaaccata gcattggatgg 300
tccaaagatc cttagctcaa gagcatggaa tagagactcc caagtatctg gctgatttgt 360

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ttgattataa aaccaagaga tttatagaag ttggaataac aaaaggattg gctgatgatt 420
acttttggaa aaagaaagaa aagctgggaa atagcatgga actgatgata ttcagctaca 480
atcaagacta ttgcttaagt aatgaatcct cattggatga ggaagggaaa gggagagatgc 540
taagcagact cacagaactt caggctgaat taagtctgaa aaacctatgg caagtctcca 600
taggagaaga agatgttgaa aagggaattg actttaaact tggacaaaca atatctagac 660
taagggatata atctgttcca gctggtttct ccaattttga aggaatgagg agctacatag 720
acaatataga cccgaaagga gcaatagaga gaaatctagc aaggatgtct cccttagtat 780
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accatgagct accagaagtt ccatataatg cctttcttct aatgtctgat gaactggggc 900
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aattaaaaat gcaogtgttt ctact 2305

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<210> 22

<211> 1982

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 22

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tttccataaa tgcacgcagc acaaaaaact agacagctac ccaatcttct cagaggatag 420
gaaaaaatca gattatcaac ccaaaacggt atcaacacag aaaaggcacc agggaggacc 480

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 gaagtccacc acatctgtac aaaagaagaa gaccaaatta ctgtttgggg gttccattct 660
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 cctgtatttt cctttattgt ggtgcttgtt tgctgtttat cattacaaag aaacgttatt 1860
 gaaaaatgct cttgttacta ct 1882

<210> 23

<211> 1844

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 23

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 ataacttttg gaaccagtgg gacaacocaga ccaatcatca gaccagcaac ccttgcccca 180
 ccaagcaaca aacgaacccg taacccatcc ccggaaaagag caaccacaag cagtgaagct 240
 gatgtcggga ggaaaaccca aaagaacacg accccgacag agataaagaa gagcgtctac 300
 aacatggtag tgaacttggg cgaattctac aaccagatga tggtcaaagc tggactcaac 360
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 gccactgatg acaagaaaac tgaattccag aagaaaagaa ataccagaga tgtcaaaaga 480
 gggaaggaag aatatagatca caacaaaaca ggaggcaact tttaacaagat ggtaagagat 540
 gataaaacca tctacttcag cctcataaga attacctttt taaaagaaga ggtgaaaaca 600
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 cattcacaga tgaatgatgt ctgtttccaa agatcaaaag cactaaaaag agttggactt 720
 gaccttcat taatcagtac ctttgcggga agcacaatcc ccagaagatc aggtgcaact 780
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gtaagaagaa tgctgtcaat gaattatgag ggacgtgatg cagatgtcaa aggaatacta 1560
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accatcccca atttcttctt tgggagggac acagcagagg attatgatga cctcgattat 1740
taaagcaaca aatatagcac tatgactgtg attgtttcaa tacgtttgga atgtgggtgt 1800
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<210> 24

<211> 1557

<212> DNA

<213> Influenza B/Vienna/1/99/ca

<400> 24

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atgtgtcagc ttcaactgtca tacttattat attcggatat attgctaaaa ttcttaccac 180
cagaataaac tgcaccaaca atgcccattg atgttgcaaa cgcatacaat gttccaggctg 240
tgaaaccttc tgcacaacaaa ggggtgacac ttcttctccc agaaccggag tggacatacc 300
cgcttttato ttgcccgggc tcaacctttc agaaagcact cctaattagc cctcatagat 360
tcggagaaac caaaggaaac tcagctccct tgataataag ggaacotttt attgctttgtg 420
gaccaaaagg atgcaaacac tttgctctaa cccattatgc agccccacca gggggatact 480
acaatggaa aagagaaagc agaacaagc tgaggcatct aatttcagtc aaattgggca 540
aaatcccaac agtagaaaac tccattttcc acatggcagc atggagcggg tccgcatgcc 600
atgatggtaa agaattggaca tatactcggag ttgatggccc tgacagtaat gcattgtcca 660
aaataaaaat tggagaagca tatactgaca cataccattc ctatgcaaac aacatcccaa 720
gaacacaaga aagtgcctgc aattgcacat ggggaaattg ttactcttat ataactgatg 780
gtcagcttc aggtattagt gaatgcagat ttcttaagat tcaagagggc cgaatataaa 840
aagaatatt tccaacagga agagtagaac atactgaaga atgcacatgc ggaattggca 900
gcaataaaac catagaaagt gcctgtagag ataacagtta cacagcaaaa agaccctttg 960
tcaaatcaaa tgtggagact gatacagcag aaataaagatt gatgtgcaca gagacttact 1020
tggacacccc cagaccagat gatggaagca taacagggcc ttgtgaatct aatggggata 1080
aaggaggtgg aggcacaaag ggaggatttg ttcatcaaag aatggcatcc aagactggaa 1140
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atgatggaga ccaatggact gacagtgatg ccttgctctc tatgtggactc atggtttcaa 1260
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cctgtattgg gatagagatg gtacatgatg gtggaagga gacttggcac tcagcagcaa 1380
cagccattta ctgttttaag ggctcaggac aactgctatg ggacactgtc acaggtgtta 1440
atatggctct gtaatggagg aatggttgag tctgtttcaa accctttgtt cctattttgt 1500
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<210> 25
 <211> 1190
 <212> DNA
 <213> Influenza B/Vienna/1/99/ca

<400> 25
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 ggtgggaaag aatttgacct agactctgcc ttggaatgga taaaaacaa aagatgctta 180
 actgatatac aaaaagcact aatttggtgcc tctatctgct ttttaaaacc caaagaccag 240
 gaaagaaaaa gaagattcat cacagagccc ctatcaggaa tgggaaacac agcaacaasa 300
 aagaaagccc tgattctagc tgagagaaaa atgagaagat gtgtgagctt tcatgaagca 360
 tttgaaatag cagaaggcca tgaaaagctca gcgctactat attgtctcat ggtcatgtac 420
 ctgaattctg gaaattatct aatgcaagta aaactaggaa cgcctctgtc tttgtgcgag 480
 aaacaagcat caccttcaca cagggctcat agcagagcag cgagatcttc agtgcccga 540
 gtgagacgag aaatgcagat ggtctcagct atgaacacag caaaaacaat gaatggaatg 600
 ggaagaggag aagacgtcca aaaactggca gaagagctgc aaagcaacat tggagtactg 660
 agatctcttg gggcaagtca aaagaatggg gaagggaattg caaaggatgt aatggaagtg 720
 ctaaagcaga gctctatggg aaattcagct cttgtgaaga aatatctata atgtctgaac 780
 catctcagat tctttcaatt tgttctttta tcttatcagc tctccatttc gtggcttgga 840
 caatagggca tttgaatcaa ataaaaagag gagttaaacat gaaaatacga ataaaaagtc 900
 caaacaaaga gacataaac agagaggtat caacttttga acacagtgac caaaaagaaa 960
 tcaggcccaa agaaacaatg aagggaagtac tctctgacaa catggaggtg tttgggtgac 1020
 acatagtaat tgaggggctt tctgcgcaag agataataaa aatgggtgaa acagtttttg 1080
 agatagaaga attgcattaa attcaatttt tactgtattt cttactatgc atttaagcaa 1140
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<210> 26
 <211> 1097
 <212> DNA
 <213> Influenza B/Vienna/1/99/ca

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 aattctggag tgctatgaaa ggctttctatg gcaaaagagcc cttgactacc ctggtcaaga 180
 ccgctctaac agactaaaga gaaaattaga gtcaagaata aagactcaca acaaaagtga 240
 gctgtgaaag aaaaaggatgt ctcttgaaga gaggaagca attggagtaa aaatgatgaa 300
 agtactccta tttatgaaac catctgctgg aattggaagg tttagcccat actatatgaa 360
 aagttctcca aatagcaact gtccgaata caattggacc gattaccctt caacaccagg 420
 gagggtgcct gatgacatag aagaagaacc agaggatgtt gatggcccaa ctgaaatagt 480
 attaagggac atgaacaaca aagatgcaag gcaaaagata aaagagggaag taacactca 540
 gaaagaaggg aagttccgtt tgacaataaa aagggaata cgtaatgtat tgtccttgag 600
 agtgttggtg aacggaacat tctcaaaaca cccaatgga tacaagtctt tatcaactct 660
 gcatagattg aatgcatatg accagagtgg aaggcttgtt gctaaaactgt ttgctactga 720
 tgactctaca gtggaggtg aagaagatgg ccatcggtac ctcaactcac tcttcagagc 780
 tcttaagtga ggacattcaa agccaattcg agcagctgaa actgcggtgg gagtctatc 840
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aagaacttta tcttttaagt aaaagaattg atgataacat attgttccac aaaacagtaa 960
 tagctaacag ctccataata gctgacatgg ttgtatcatt atcattatta gaaacattgt 1020
 atgaaatgaa ggaatgtggtt gaagtgtaca gcaggcagtg cttgtgaatt taaaataaaa 1080
 atcctcttgt tactact 1097

<210> 27

<211> 770

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 27

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Glu Ala Lys Thr Val Leu Lys Gln Thr Thr Val Asp Gln Tyr Asn Ile
 20 25 30

Ile Arg Lys Phe Asn Thr Ser Arg Ile Glu Lys Asn Pro Ser Leu Arg
 35 40 45

Met Lys Trp Ala Met Cys Ser Asn Phe Pro Leu Ala Leu Thr Lys Gly
 50 55 60

Asp Met Ala Asn Arg Ile Pro Leu Glu Tyr Lys Gly Ile Gln Leu Lys
 65 70 75 80

Thr Asn Ala Glu Asp Ile Gly Thr Lys Gly Gln Met Cys Ser Ile Ala
 85 90 95

Ala Val Thr Trp Trp Asn Thr Tyr Gly Pro Ile Gly Asp Thr Glu Gly
 100 105 110

Phe Glu Lys Val Tyr Glu Ser Phe Phe Leu Arg Lys Met Arg Leu Asp
 115 120 125

Asn Ala Thr Trp Gly Arg Ile Thr Phe Gly Pro Val Glu Arg Val Arg
 130 135 140

Lys Arg Val Leu Leu Asn Pro Leu Thr Lys Glu Met Pro Pro Asp Glu
 145 150 155 160

Ala Ser Asn Val Ile Met Glu Ile Leu Phe Pro Lys Glu Ala Gly Ile
 165 170 175

Pro Arg Glu Ser Thr Trp Ile His Arg Glu Leu Ile Lys Glu Lys Arg
 180 185 190

Glu Lys Leu Lys Gly Thr Met Ile Thr Pro Ile Val Leu Ala Tyr Met

195	200	205
Leu Glu Arg Glu Leu Val Ala Arg Arg Arg Phe Leu Pro Val Ala Gly		
210	215	220
Ala Thr Ser Ala Glu Phe Ile Glu Met Leu His Cys Leu Gln Gly Glu		
225	230	235
Asn Trp Arg Gln Ile Tyr His Pro Gly Gly Asn Lys Leu Thr Glu Ser		
245	250	255
Arg Ser Gln Ser Met Ile Val Ala Cys Arg Lys Ile Ile Arg Arg Ser		
260	265	270
Ile Val Ala Ser Asn Pro Leu Glu Leu Ala Val Glu Ile Ala Asn Lys		
275	280	285
Thr Val Ile Asp Thr Glu Pro Leu Lys Ser Cys Leu Thr Ala Ile Asp		
290	295	300
Gly Gly Asp Val Ala Cys Asp Ile Ile Arg Ala Ala Leu Gly Leu Lys		
305	310	315
Ile Arg Gln Arg Gln Arg Phe Gly Arg Leu Glu Leu Lys Arg Ile Ser		
325	330	335
Gly Arg Gly Phe Lys Asn Asp Glu Glu Ile Leu Ile Gly Asn Gly Thr		
340	345	350
Ile Gln Lys Ile Gly Ile Trp Asp Gly Glu Glu Glu Phe His Val Arg		
355	360	365
Cys Gly Glu Cys Arg Gly Ile Leu Lys Lys Ser Lys Met Arg Met Glu		
370	375	380
Lys Leu Leu Ile Asn Ser Ala Lys Lys Glu Asp Met Lys Asp Leu Ile		
385	390	395
Ile Leu Cys Met Val Phe Ser Gln Asp Thr Arg Met Phe Gln Gly Val		
405	410	415
Arg Gly Glu Ile Asn Phe Leu Asn Arg Ala Gly Gln Leu Leu Ser Pro		
420	425	430
Met Tyr Gln Leu Gln Arg Tyr Phe Leu Asn Arg Ser Asn Asp Leu Phe		
435	440	445
Asp Gln Trp Gly Tyr Glu Glu Ser Pro Lys Ala Ser Glu Leu His Gly		

450 455 460
 Ile Asn Glu Leu Met Asn Ala Ser Asp Tyr Thr Leu Lys Gly Val Val
 465 470 475 480
 Val Thr Lys Asn Val Ile Asp Asp Phe Ser Ser Thr Glu Thr Glu Lys
 485 490 495
 Val Ser Ile Thr Lys Asn Leu Ser Leu Ile Lys Arg Thr Gly Glu Val
 500 505 510
 Ile Met Gly Ala Asn Asp Val Ser Glu Leu Glu Ser Gln Ala Gln Leu
 515 520 525
 Met Ile Thr Tyr Asp Thr Pro Lys Met Trp Glu Met Gly Thr Thr Lys
 530 535 540
 Glu Leu Val Gln Asn Thr Tyr Gln Trp Val Leu Lys Asn Leu Val Thr
 545 550 555 560
 Leu Lys Ala Gln Phe Leu Leu Gly Lys Glu Asp Met Phe Gln Trp Asp
 565 570 575
 Ala Phe Glu Ala Phe Glu Ser Ile Ile Pro Gln Lys Met Ala Gly Gln
 580 585 590
 Tyr Ser Gly Phe Ala Arg Ala Val Leu Lys Gln Met Arg Asp Gln Glu
 595 600 605
 Val Met Lys Thr Asp Gln Phe Ile Lys Leu Leu Pro Phe Cys Phe Ser
 610 615 620
 Pro Pro Lys Leu Arg Ser Asn Gly Glu Pro Tyr Gln Phe Leu Arg Leu
 625 630 635 640
 Val Leu Lys Gly Gly Gly Glu Asn Phe Ile Glu Val Arg Lys Gly Ser
 645 650 655
 Pro Leu Phe Ser Tyr Asn Pro Gln Thr Glu Val Leu Thr Ile Cys Gly
 660 665 670
 Arg Met Met Ser Leu Lys Gly Lys Ile Glu Asp Glu Glu Arg Asn Arg
 675 680 685
 Ser Met Gly Asn Ala Val Leu Ala Gly Phe Leu Val Ser Gly Lys Tyr
 690 695 700
 Asp Pro Asp Leu Gly Asp Phe Lys Thr Ile Glu Glu Leu Glu Lys Leu

705	710	715	720
Lys Pro Gly Glu Lys Ala Asn Ile Leu Leu Tyr Gln Gly Lys Pro Val			
725		730	735
Lys Val Val Lys Arg Lys Arg Tyr Ser Ala Leu Ser Asn Asp Ile Ser			
740	745		750
Gln Gly Ile Lys Arg Gln Arg Met Thr Val Glu Ser Met Gly Trp Ala			
755	760		765
Leu Ser			
770			

<210> 28
 <211> 752
 <212> PRT
 <213> Influenza B/Vienna/1/99/ca

<400> 28
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Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Val Pro Pro Tyr Ser His
20 25 30
Gly Thr Gly Thr Gly His Thr Ile Asp Thr Val Ile Arg Thr His Glu
35 40 45
Tyr Ser Asn Lys Gly Lys Gln Tyr Val Ser Asp Ile Thr Gly Cys Thr
50 55 60
Met Val Asp Pro Thr Asn Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
65 70 75 80
Ala Tyr Ala Gln Leu Asp Cys Val Leu Glu Ala Leu Asp Arg Met Asp
85 90 95
Glu Glu His Pro Gly Leu Phe Gln Ala Ala Ser Gln Asn Ala Met Glu
100 105 110
Ala Leu Met Val Thr Thr Val Asp Lys Leu Thr Gln Gly Arg Gln Thr
115 120 125
Phe Asp Trp Thr Val Cys Arg Asn Gln Pro Ala Ala Thr Ala Leu Asn
130 135 140

Thr Thr Ile Thr Ser Phe Arg Leu Asn Asp Leu Asn Gly Ala Asp Lys
 145 150 155 160
 Gly Gly Leu Val Pro Phe Cys Gln Asp Ile Ile Asp Ser Leu Asp Lys
 165 170 175
 Pro Glu Met Thr Phe Phe Ser Val Lys Asn Ile Lys Lys Lys Phe Pro
 180 185 190
 Ala Lys Asn Arg Lys Gly Phe Leu Ile Lys Arg Ile Pro Met Lys Val
 195 200 205
 Lys Asp Arg Ile Ser Arg Val Glu Tyr Ile Lys Arg Ala Leu Ser Leu
 210 215 220
 Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
 225 230 235 240
 Ile Ala Thr Ala Gly Ile Gln Ile Arg Gly Phe Val Leu Val Val Glu
 245 250 255
 Asn Leu Ala Lys Asn Ile Cys Glu Asn Leu Glu Gln Ser Gly Leu Pro
 260 265 270
 Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ser Asn Ala Val Ala Lys
 275 280 285
 Met Leu Ser Asn Cys Pro Pro Gly Gly Ile Ser Met Thr Val Thr Gly
 290 295 300
 Asp Asn Thr Lys Trp Asn Glu Cys Leu Asn Pro Arg Val Phe Leu Ala
 305 310 315 320
 Met Thr Glu Arg Ile Thr Arg Asp Ser Pro Ile Trp Phe Arg Asp Phe
 325 330 335
 Cys Ser Ile Ala Pro Val Leu Phe Ser Asn Lys Ile Ala Arg Leu Gly
 340 345 350
 Lys Gly Phe Met Ile Thr Ser Lys Thr Lys Arg Leu Lys Ala Gln Ile
 355 360 365
 Pro Cys Pro Asp Leu Phe Ser Ile Pro Leu Glu Arg Tyr Asn Glu Glu
 370 375 380
 Thr Arg Ala Lys Leu Lys Lys Leu Lys Pro Phe Phe Asn Glu Glu Gly
 385 390 395 400

Thr Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu
 405 410 415

Ser Thr Val Leu Gly Val Ala Ala Leu Gly Ile Lys Asn Ile Gly Asn
 420 425 430

Lys Glu Tyr Leu Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala Leu
 435 440 445

Phe Val Asn Ala Lys Asp Glu Glu Thr Cys Met Glu Gly Ile Asn Asp
 450 455 460

Phe Tyr Arg Thr Cys Lys Leu Leu Gly Ile Asn Met Ser Lys Lys Lys
 465 470 475 480

Ser Tyr Cys Asn Glu Thr Gly Met Phe Glu Phe Thr Ser Met Phe Tyr
 485 490 495

Arg Asp Gly Phe Val Ser Asn Phe Ala Met Glu Ile Pro Ser Phe Gly
 500 505 510

Val Ala Gly Val Asn Glu Ser Ala Asp Met Ala Ile Gly Met Thr Ile
 515 520 525

Ile Lys Asn Asn Met Ile Asn Asn Gly Met Gly Pro Ala Thr Ala Gln
 530 535 540

Thr Ala Ile Gln Leu Phe Ile Ala Asp Tyr Arg Tyr Thr Tyr Lys Cys
 545 550 555 560

His Arg Gly Asp Ser Lys Val Glu Gly Lys Arg Met Lys Ile Ile Lys
 565 570 575

Glu Leu Trp Glu Asn Thr Lys Gly Arg Asp Gly Leu Leu Val Ala Asp
 580 585 590

Gly Gly Pro Asn Ile Tyr Asn Leu Arg Asn Leu His Ile Pro Glu Ile
 595 600 605

Val Leu Lys Tyr Asn Leu Met Asp Pro Glu Tyr Lys Gly Arg Leu Leu
 610 615 620

His Pro Gln Asn Pro Phe Val Gly His Leu Ser Ile Glu Gly Ile Lys
 625 630 635 640

Glu Ala Asp Ile Thr Pro Ala His Gly Pro Val Lys Lys Met Asp Tyr
 645 650 655

Asp Ala Val Ser Gly Thr His Ser Trp Arg Thr Lys Arg Asn Arg Ser
660 665 670

Ile Leu Asn Thr Asp Gln Arg Asn Met Ile Leu Glu Glu Gln Cys Tyr
675 680 685

Ala Lys Cys Cys Asn Leu Phe Glu Ala Cys Phe Asn Ser Ala Ser Tyr
690 695 700

Arg Lys Pro Val Gly Gln His Ser Met Leu Glu Ala Met Ala His Arg
705 710 715 720

Leu Arg Met Asp Ala Arg Leu Asp Tyr Glu Ser Gly Arg Met Ser Lys
725 730 735

Asp Asp Phe Glu Lys Ala Met Ala His Leu Gly Glu Ile Gly Tyr Thr
740 745 750

<210> 29

<211> 726

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 29

Met Asp Thr Phe Ile Thr Arg Asn Phe Gln Thr Thr Ile Ile Gln Lys
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Ala Lys Asn Thr Met Ala Glu Phe Ser Glu Asp Pro Glu Leu Gln Pro
20 25 30

Ala Met Leu Phe Asn Ile Cys Val His Leu Glu Val Cys Tyr Val Ile
35 40 45

Ser Asp Met Asn Phe Leu Asp Glu Glu Gly Lys Ala Tyr Thr Ala Leu
50 55 60

Glu Gly Gln Gly Lys Glu Gln Asn Leu Arg Pro Gln Tyr Glu Val Ile
65 70 75 80

Glu Gly Met Pro Arg Thr Ile Ala Trp Met Val Gln Arg Ser Leu Ala
85 90 95

Gln Glu His Gly Ile Glu Thr Pro Lys Tyr Leu Ala Asp Leu Phe Asp
100 105 110

Tyr Lys Thr Lys Arg Phe Ile Glu Val Gly Ile Thr Lys Gly Leu Ala
 115 120 125
 Asp Asp Tyr Phe Trp Lys Lys Lys Glu Lys Leu Gly Asn Ser Met Glu
 130 135 140
 Leu Met Ile Phe Ser Tyr Asn Gln Asp Tyr Ser Leu Ser Asn Glu Ser
 145 150 155 160
 Ser Leu Asp Glu Glu Gly Lys Gly Arg Val Leu Ser Arg Leu Thr Glu
 165 170 175
 Leu Gln Ala Glu Leu Ser Leu Lys Asn Leu Trp Gln Val Leu Ile Gly
 180 185 190
 Glu Glu Asp Val Glu Lys Gly Ile Asp Phe Lys Leu Gly Gln Thr Ile
 195 200 205
 Ser Arg Leu Arg Asp Ile Ser Val Pro Ala Gly Phe Ser Asn Phe Glu
 210 215 220
 Gly Met Arg Ser Tyr Ile Asp Asn Ile Asp Pro Lys Gly Ala Ile Glu
 225 230 235 240
 Arg Asn Leu Ala Arg Met Ser Pro Leu Val Ser Val Thr Pro Lys Lys
 245 250 255
 Leu Lys Trp Glu Asp Leu Arg Pro Ile Gly Pro His Ile Tyr Asn His
 260 265 270
 Glu Leu Pro Glu Val Pro Tyr Asn Ala Phe Leu Leu Met Ser Asp Glu
 275 280 285
 Leu Gly Leu Ala Asn Met Thr Glu Gly Lys Ser Lys Lys Pro Lys Thr
 290 295 300
 Leu Ala Lys Glu Cys Leu Glu Lys Tyr Ser Thr Leu Arg Asp Gln Thr
 305 310 315 320
 Asp Pro Ile Leu Ile Met Lys Ser Glu Lys Ala Asn Glu Asn Phe Leu
 325 330 335
 Trp Lys Leu Trp Arg Asp Cys Val Asn Thr Ile Ser Asn Glu Glu Met
 340 345 350
 Ser Asn Glu Leu Gln Lys Thr Asn Tyr Ala Lys Trp Ala Thr Gly Asp
 355 360 365

Gly Leu Thr Tyr Gln Lys Ile Met Lys Glu Val Ala Ile Asp Asp Glu
 370 375 380
 Thr Met Cys Gln Glu Glu Pro Lys Ile Pro Asn Lys Cys Arg Val Ala
 385 390 395 400
 Ala Trp Val Gln Thr Glu Met Asn Leu Leu Ser Thr Leu Thr Ser Lys
 405 410 415
 Lys Ala Leu Asp Leu Pro Glu Ile Gly Pro Asp Val Ala Pro Val Glu
 420 425 430
 His Val Gly Ser Glu Arg Arg Lys Tyr Phe Val Asn Glu Ile Asn Tyr
 435 440 445
 Cys Lys Ala Ser Thr Val Met Met Lys Tyr Val Leu Phe His Thr Ser
 450 455 460
 Leu Leu Asn Glu Ser Asn Ala Ser Met Gly Lys Tyr Lys Val Ile Pro
 465 470 475 480
 Ile Thr Asn Arg Val Val Asn Glu Lys Gly Glu Ser Phe Asp Met Leu
 485 490 495
 Tyr Gly Leu Ala Val Lys Gly Gln Ser His Leu Arg Gly Asp Thr Asp
 500 505 510
 Val Val Thr Val Val Thr Phe Glu Phe Ser Ser Thr Asp Pro Arg Val
 515 520 525
 Asp Ser Gly Lys Trp Pro Lys Tyr Thr Val Phe Arg Ile Gly Ser Leu
 530 535 540
 Phe Val Ser Gly Arg Glu Lys Ser Val Tyr Leu Tyr Cys Arg Val Asn
 545 550 555 560
 Gly Thr Asn Lys Ile Gln Met Lys Trp Gly Met Glu Ala Arg Arg Cys
 565 570 575
 Leu Leu Gln Ser Met Gln Gln Met Glu Ala Ile Val Glu Gln Glu Ser
 580 585 590
 Ser Ile Gln Gly Tyr Asp Met Thr Lys Ala Cys Phe Lys Gly Asp Arg
 595 600 605
 Val Asn Ser Pro Lys Thr Phe Ser Ile Gly Thr Gln Glu Gly Lys Leu
 610 615 620

Val Lys Gly Ser Phe Gly Lys Ala Leu Arg Val Ile Phe Thr Lys Cys
 625 630 635 640

Leu Met His Tyr Val Phe Gly Asn Ala Gln Leu Glu Gly Phe Ser Ala
 645 650 655

Glu Ser Arg Arg Leu Leu Leu Leu Ile Gln Ala Leu Lys Asp Arg Lys
 660 665 670

Gly Pro Trp Val Phe Asp Leu Glu Gly Met Tyr Ser Gly Ile Glu Glu
 675 680 685

Cys Ile Ser Asn Asn Pro Trp Val Ile Gln Ser Ala Tyr Trp Phe Asn
 690 695 700

Glu Trp Leu Gly Phe Glu Lys Glu Gly Ser Lys Val Leu Glu Ser Val
 705 710 715 720

Asp Glu Ile Met Asp Glu
 725

<210> 30

<211> 584

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 30

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
 1 5 10 15

Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
 20 25 30

Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Ala Ile Pro Leu Thr
 35 40 45

Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Lys Thr
 50 55 60

Arg Gly Lys Leu Cys Pro Thr Cys Leu Asn Cys Thr Asp Leu Asp Val
 65 70 75 80

Ala Leu Gly Arg Pro Met Cys Val Gly Ile Thr Pro Ser Ala Lys Ala
 85 90 95

Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile

100										105										110										
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	Gly															
		115						120						125																
Tyr	Glu	Lys	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asn	Thr	Glu	Lys															
		130				135					140																			
Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn															
		145				150				155				160																
Ala	Thr	Ser	Lys	Ser	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro															
			165						170					175																
Arg	Asp	Asn	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val	Pro															
		180						185					190																	
His	Ile	Cys	Thr	Lys	Glu	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	His															
		195					200					205																		
Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Asn	Leu	Tyr	Gly	Asp	Ser	Asn	Pro															
		210				215					220																			
Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Ile	Thr	Thr	His	Tyr	Val	Ser															
		225				230				235				240																
Gln	Ile	Gly	Gly	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	Gln															
			245					250					255																	
Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Pro	Gly	Lys	Thr															
		260					265					270																		
Gly	Thr	Ile	Val	Tyr	Gln	Arg	Gly	Ile	Leu	Leu	Pro	Gln	Lys	Val	Trp															
		275				280					285																			
Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu	Ile															
		290				295					300																			
Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys	Ser															
		305				310				315			320																	
Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys	Pro															
			325					330				335																		
Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr	Arg															
		340					345					350																		
Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile	Ala															

355	360	365
Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly		
370	375	380
Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Asp Leu Lys		
385	390	400
Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu		
405	410	415
Ser Glu Leu Glu Val Asn Asn Leu Gln Arg Leu Ser Gly Ala Met Asp		
420	425	430
Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu		
435	440	445
Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser		
450	455	460
Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu		
465	470	475
Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn		
485	490	495
Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg		
500	505	510
Ile Ala Ala Gly Thr Phe Asn Ala Glu Glu Phe Ser Leu Pro Thr Phe		
515	520	525
Asp Ser Leu Asn Ile Thr Ala Ala Ser Leu Asn Asp Asp Gly Leu Asp		
530	535	540
Asn His Thr Ile Leu Leu Tyr Tyr Ser Thr Ala Ala Ser Ser Leu Ala		
545	550	555
Val Thr Leu Met Ile Ala Ile Phe Ile Val Tyr Met Ile Ser Arg Asp		
565	570	575
Asn Val Ser Cys Ser Ile Cys Leu		
580		

<210> 31
 <211> 560
 <212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 31

Met Ser Asn Met Asp Ile Asp Gly Ile Asn Thr Gly Thr Ile Asp Lys
 1 5 10 15

Thr Pro Glu Glu Ile Thr Phe Gly Thr Ser Gly Thr Thr Arg Pro Ile
 20 25 30

Ile Arg Pro Ala Thr Leu Ala Pro Pro Ser Asn Lys Arg Thr Arg Asn
 35 40 45

Pro Ser Pro Glu Arg Ala Thr Thr Ser Ser Glu Ala Asp Val Gly Arg
 50 55 60

Lys Thr Gln Lys Lys Gln Thr Pro Thr Glu Ile Lys Lys Ser Val Tyr
 65 70 75 80

Asn Met Val Val Lys Leu Gly Glu Phe Tyr Asn Gln Met Met Val Lys
 85 90 95

Ala Gly Leu Asn Asp Asp Met Glu Arg Asn Leu Ile Gln Asn Ala His
 100 105 110

Ala Val Glu Arg Ile Leu Leu Ala Ala Thr Asp Asp Lys Lys Thr Glu
 115 120 125

Phe Gln Lys Lys Lys Asn Thr Arg Asp Val Lys Glu Gly Lys Glu Glu
 130 135 140

Ile Asp His Asn Lys Thr Gly Gly Thr Phe Tyr Lys Met Val Arg Asp
 145 150 155 160

Asp Lys Thr Ile Tyr Phe Ser Pro Ile Arg Ile Thr Phe Leu Lys Glu
 165 170 175

Glu Val Lys Thr Met Tyr Lys Thr Thr Met Gly Ser Asp Gly Phe Ser
 180 185 190

Gly Leu Asn His Ile Met Ile Gly His Ser Gln Met Asn Asp Val Cys
 195 200 205

Phe Gln Arg Ser Lys Ala Leu Lys Arg Val Gly Leu Asp Pro Ser Leu
 210 215 220

Ile Ser Thr Phe Ala Gly Ser Thr Ile Pro Arg Arg Ser Gly Ala Thr
 225 230 235 240

Gly Val Ala Ile Lys Gly Gly Gly Thr Leu Val Ala Glu Ala Ile Arg
 245 250 255

Phe Ile Gly Arg Ala Met Ala Asp Arg Gly Leu Leu Arg Asp Ile Lys
 260 265 270

Ala Lys Thr Ala Tyr Glu Lys Ile Leu Leu Asn Leu Lys Asn Lys Cys
 275 280 285

Ser Ala Pro Gln Gln Lys Ala Leu Val Asp Gln Val Ile Gly Ser Arg
 290 295 300

Asn Pro Gly Ile Ala Asp Ile Glu Asp Leu Thr Leu Leu Ala Arg Ser
 305 310 315 320

Met Val Val Val Arg Pro Ser Val Ala Ser Lys Val Val Leu Pro Ile
 325 330 335

Ser Ile Tyr Ala Lys Ile Pro Gln Leu Gly Phe Asn Val Glu Glu Tyr
 340 345 350

Ser Met Val Gly Tyr Glu Ala Met Ala Leu Tyr Asn Met Ala Thr Pro
 355 360 365

Val Ser Ile Leu Arg Met Gly Asp Asp Ala Lys Asp Lys Ser Gln Leu
 370 375 380

Phe Phe Met Ser Cys Phe Gly Ala Ala Tyr Glu Asp Leu Arg Val Leu
 385 390 395 400

Ser Ala Leu Thr Gly Thr Glu Phe Lys Pro Arg Ser Ala Leu Lys Cys
 405 410 415

Lys Gly Phe His Val Pro Ala Lys Glu Gln Val Glu Gly Met Gly Ala
 420 425 430

Ala Leu Met Ser Ile Lys Leu Gln Phe Trp Ala Pro Met Thr Arg Ser
 435 440 445

Gly Gly Asn Glu Val Gly Gly Asp Gly Gly Ser Gly Gln Ile Ser Cys
 450 455 460

Ser Pro Val Phe Ala Val Glu Arg Pro Ile Ala Leu Ser Lys Gln Ala
 465 470 475 480

Val Arg Arg Met Leu Ser Met Asn Ile Glu Gly Arg Asp Ala Asp Val
 485 490 495

Lys Gly Asn Leu Leu Lys Met Met Asn Asp Ser Met Ala Lys Lys Thr
 500 505 510

Ser Gly Asn Ala Phe Ile Gly Lys Lys Met Phe Gln Ile Ser Asp Lys
 515 520 525

Asn Lys Thr Asn Pro Val Glu Ile Pro Ile Lys Gln Thr Ile Pro Asn
 530 535 540

Phe Phe Phe Gly Arg Asp Thr Ala Glu Asp Tyr Asp Asp Leu Asp Tyr
 545 550 555 560

<210> 32

<211> 100

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 32

Met Asn Asn Ala Thr Phe Asn Tyr Thr Asn Val Asn Pro Ile Pro His
 1 5 10 15

Ile Arg Gly Ser Val Ile Ile Thr Ile Cys Val Ser Phe Thr Val Ile
 20 25 30

Leu Ile Ile Phe Gly Tyr Ile Ala Lys Ile Phe Thr Asn Arg Asn Asn
 35 40 45

Cys Thr Asn Asn Ala Ile Gly Leu Cys Lys Arg Ile Lys Cys Ser Gly
 50 55 60

Cys Glu Pro Phe Cys Asn Lys Arg Gly Asp Thr Ser Ser Pro Arg Thr
 65 70 75 80

Gly Val Asp Ile Pro Ala Phe Ile Leu Pro Gly Leu Asn Leu Ser Glu
 85 90 95

Ser Thr Pro Asn
 100

<210> 33

<211> 466

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 33

Met Leu Pro Ser Thr Ile Gln Thr Leu Thr Leu Phe Leu Thr Ser Gly
 1 5 10 15

Gly Val Leu Leu Ser Leu Tyr Val Ser Ala Ser Leu Ser Tyr Leu Leu
 20 25 30

Tyr Ser Asp Ile Leu Leu Lys Phe Ser Pro Thr Glu Ile Thr Ala Pro
 35 40 45

Thr Met Pro Leu Asp Cys Ala Asn Ala Ser Asn Val Gln Ala Val Asn
 50 55 60

Arg Ser Ala Thr Lys Gly Val Thr Leu Leu Leu Pro Glu Pro Glu Trp
 65 70 75 80

Thr Tyr Pro Arg Leu Ser Cys Pro Gly Ser Thr Phe Gln Lys Ala Leu
 85 90 95

Leu Ile Ser Pro His Arg Phe Gly Glu Thr Lys Gly Asn Ser Ala Pro
 100 105 110

Leu Ile Ile Arg Glu Pro Phe Ile Ala Cys Gly Pro Lys Glu Cys Lys
 115 120 125

His Phe Ala Leu Thr His Tyr Ala Ala Gln Pro Gly Gly Tyr Tyr Asn
 130 135 140

Gly Thr Arg Glu Asp Arg Asn Lys Leu Arg His Leu Ile Ser Val Lys
 145 150 155 160

Leu Gly Lys Ile Pro Thr Val Glu Asn Ser Ile Phe His Met Ala Ala
 165 170 175

Trp Ser Gly Ser Ala Cys His Asp Gly Lys Glu Trp Thr Tyr Ile Gly
 180 185 190

Val Asp Gly Pro Asp Ser Asn Ala Leu Leu Lys Ile Lys Tyr Gly Glu
 195 200 205

Ala Tyr Thr Asp Thr Tyr His Ser Tyr Ala Asn Asn Ile Leu Arg Thr
 210 215 220

Gln Glu Ser Ala Cys Asn Cys Ile Gly Gly Asn Cys Tyr Leu Met Ile
 225 230 235 240

Thr Asp Gly Ser Ala Ser Gly Ile Ser Glu Cys Arg Phe Leu Lys Ile

245										250					255				
Gln	Glu	Gly	Arg	Ile	Ile	Lys	Glu	Ile	Phe	Pro	Thr	Gly	Arg	Val	Glu				
			260						265					270					
His	Thr	Glu	Glu	Cys	Thr	Cys	Gly	Phe	Ala	Ser	Asn	Lys	Thr	Ile	Glu				
		275					280						285						
Cys	Ala	Cys	Arg	Asp	Asn	Ser	Tyr	Thr	Ala	Lys	Arg	Pro	Phe	Val	Lys				
		290					295					300							
Leu	Asn	Val	Glu	Thr	Asp	Thr	Ala	Glu	Ile	Arg	Leu	Met	Cys	Thr	Glu				
305					310					315					320				
Thr	Tyr	Leu	Asp	Thr	Pro	Arg	Pro	Asp	Asp	Gly	Ser	Ile	Thr	Gly	Pro				
			325						330					335					
Cys	Glu	Ser	Asn	Gly	Asp	Lys	Gly	Ser	Gly	Gly	Ile	Lys	Gly	Gly	Phe				
			340					345						350					
Val	His	Gln	Arg	Met	Ala	Ser	Lys	Thr	Gly	Arg	Trp	Tyr	Ser	Arg	Thr				
		355						360						365					
Met	Ser	Lys	Thr	Lys	Arg	Met	Gly	Met	Gly	Leu	Tyr	Val	Lys	Tyr	Asp				
		370					375					380							
Gly	Asp	Pro	Trp	Thr	Asp	Ser	Asp	Ala	Leu	Ala	Leu	Ser	Gly	Val	Met				
385					390					395					400				
Val	Ser	Met	Glu	Glu	Pro	Gly	Trp	Tyr	Ser	Phe	Gly	Phe	Glu	Ile	Lys				
			405						410					415					
Asp	Lys	Lys	Cys	Asp	Val	Pro	Cys	Ile	Gly	Ile	Glu	Met	Val	His	Asp				
			420					425						430					
Gly	Gly	Lys	Glu	Thr	Trp	His	Ser	Ala	Ala	Thr	Ala	Ile	Tyr	Cys	Leu				
		435						440						445					
Met	Gly	Ser	Gly	Gln	Leu	Leu	Trp	Asp	Thr	Val	Thr	Gly	Val	Asn	Met				
		450					455						460						
Ala	Leu																		
465																			

<210> 34
 <211> 248
 <212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 34

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Met Ser Leu Phe Gly Asp Thr Ile Ala Tyr Leu Leu Ser Leu Thr Glu
 1             5             10             15

Asp Gly Glu Gly Lys Ala Glu Leu Ala Glu Lys Leu His Cys Trp Phe
          20             25             30

Gly Gly Lys Glu Phe Asp Leu Asp Ser Ala Leu Glu Trp Ile Lys Asn
          35             40             45

Lys Arg Cys Leu Thr Asp Ile Gln Lys Ala Leu Ile Gly Ala Ser Ile
          50             55             60

Cys Phe Leu Lys Pro Lys Asp Gln Glu Arg Lys Arg Arg Phe Ile Thr
          65             70             75             80

Glu Pro Leu Ser Gly Met Gly Thr Thr Ala Thr Lys Lys Lys Gly Leu
          85             90             95

Ile Leu Ala Glu Arg Lys Met Arg Arg Cys Val Ser Phe His Glu Ala
          100             105             110

Phe Glu Ile Ala Glu Gly His Glu Ser Ser Ala Leu Leu Tyr Cys Leu
          115             120             125

Met Val Met Tyr Leu Asn Pro Gly Asn Tyr Ser Met Gln Val Lys Leu
          130             135             140

Gly Thr Leu Cys Ala Leu Cys Glu Lys Gln Ala Ser His Ser His Arg
          145             150             155             160

Ala His Ser Arg Ala Ala Arg Ser Ser Val Pro Gly Val Arg Arg Glu
          165             170             175

Met Gln Met Val Ser Ala Met Asn Thr Ala Lys Thr Met Asn Gly Met
          180             185             190

Gly Lys Gly Glu Asp Val Gln Lys Leu Ala Glu Glu Leu Gln Ser Asn
          195             200             205

Ile Gly Val Leu Arg Ser Leu Gly Ala Ser Gln Lys Asn Gly Glu Gly
          210             215             220

Ile Ala Lys Asp Val Met Glu Val Leu Lys Gln Ser Ser Met Gly Asn
          225             230             235             240

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Ser Ala Leu Val Lys Lys Tyr Leu
245

<210> 35
<211> 109
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 35
Met Leu Glu Pro Phe Gln Ile Leu Ser Ile Cys Ser Phe Ile Leu Ser
1 5 10 15
Ala Leu His Phe Val Ala Trp Thr Ile Gly His Leu Asn Gln Ile Lys
20 25 30
Arg Gly Val Asn Met Lys Ile Arg Ile Lys Ser Pro Asn Lys Glu Thr
35 40 45
Ile Asn Arg Glu Val Ser Ile Leu Arg His Ser Tyr Gln Lys Glu Ile
50 55 60
Gln Ala Lys Glu Thr Met Lys Glu Val Leu Ser Asp Asn Met Glu Val
65 70 75 80
Leu Gly Asp His Ile Val Ile Glu Gly Leu Ser Ala Glu Glu Ile Ile
85 90 95
Lys Met Gly Glu Thr Val Leu Glu Ile Glu Glu Leu His
100 105

<210> 36
<211> 281
<212> PRT
<213> Influenza B/Vienna/1/99/ca

<400> 36
Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Val Gly Pro Gly Ala
1 5 10 15
Thr Asn Ala Thr Ile Asn Phe Glu Thr Gly Ile Leu Glu Cys Tyr Glu
20 25 30
Arg Leu Ser Trp Gln Arg Ala Leu Asp Tyr Pro Gly Gln Asp Arg Leu
35 40 45
Asn Arg Leu Lys Arg Lys Leu Glu Ser Arg Ile Lys Thr His Asn Lys

50	55	60
Ser Glu Pro Glu Ser Lys Arg Met Ser Leu Glu Glu Arg Lys Ala Ile		
65	70	75 80
Gly Val Lys Met Met Lys Val Leu Leu Phe Met Asn Pro Ser Ala Gly		
	85	90 95
Ile Glu Gly Phe Glu Pro Tyr Tyr Met Lys Ser Ser Ser Asn Ser Asn		
	100	105 110
Cys Pro Lys Tyr Asn Trp Thr Asp Tyr Pro Ser Thr Pro Gly Arg Cys		
	115	120 125
Leu Asp Asp Ile Glu Glu Glu Pro Glu Asp Val Asp Gly Pro Thr Glu		
	130	135 140
Ile Val Leu Arg Asp Met Asn Asn Lys Asp Ala Arg Gln Lys Ile Lys		
	145	150 155 160
Glu Glu Val Asn Thr Gln Lys Glu Gly Lys Phe Arg Leu Thr Ile Lys		
	165	170 175
Arg Asp Ile Arg Asn Val Leu Ser Leu Arg Val Leu Val Asn Gly Thr		
	180	185 190
Phe Leu Lys His Pro Asn Gly Tyr Lys Ser Leu Ser Thr Leu His Arg		
	195	200 205
Leu Asn Ala Tyr Asp Gln Ser Gly Arg Leu Val Ala Lys Leu Val Ala		
	210	215 220
Thr Asp Asp Leu Thr Val Glu Asp Glu Glu Asp Gly His Arg Ile Leu		
	225	230 235 240
Asn Ser Leu Phe Glu Arg Leu Asn Glu Gly His Ser Lys Pro Ile Arg		
	245	250 255
Ala Ala Glu Thr Ala Val Gly Val Leu Ser Gln Phe Gly Gln Glu His		
	260	265 270
Arg Leu Ser Pro Glu Glu Gly Asp Asn		
	275	280

<210> 37

<211> 122

<212> PRT

<213> Influenza B/Vienna/1/99/ca

<400> 37

Met Ala Asn Asn Ile Thr Thr Thr Gln Ile Glu Trp Arg Met Lys Lys
 1 5 10 15

Met Ala Ile Gly Ser Ser Thr His Ser Ser Ser Val Leu Met Lys Asp
 20 25 30

Ile Gln Ser Gln Phe Glu Gln Leu Lys Leu Arg Trp Glu Ser Tyr Pro
 35 40 45

Asn Leu Val Lys Ser Thr Asp Tyr His Gln Lys Arg Glu Thr Ile Lys
 50 55 60

Leu Val Thr Glu Glu Leu Tyr Leu Leu Ser Lys Arg Ile Asp Asp Asn
 65 70 75 80

Ile Leu Phe His Lys Thr Val Ile Ala Asn Ser Ser Ile Ile Ala Asp
 85 90 95

Met Val Val Ser Leu Ser Leu Leu Glu Thr Leu Tyr Glu Met Lys Asp
 100 105 110

Val Val Glu Val Tyr Ser Arg Gln Cys Leu
 115 120